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**Determination of the ultimate aerobic  
biodegradability and disintegration of  
plastic materials under controlled  
composting conditions — Method by  
analysis of evolved carbon dioxide**

*Évaluation de la biodégradabilité aérobie ultime et de désintégration des  
matériaux plastiques dans des conditions contrôlées de compostage —  
Méthode par analyse du dioxyde de carbone libéré*



## Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 14855 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Annexes A to E of this International Standard are for information only.

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International Organization for Standardization  
Case postale 56 • CH-1211 Genève 20 • Switzerland  
Internet iso@iso.ch

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# Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide

**WARNING** — Sewage, activated sludge, soil and compost may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

## 1 Scope

This International Standard specifies a method for the determination of the ultimate aerobic biodegradability of plastics, based on organic compounds, under controlled composting conditions by measurement of the amount of carbon dioxide evolved and the degree of disintegration of the plastic at the end of the test. This method is designed to simulate typical aerobic composting conditions for the organic fraction of solid mixed municipal waste. The test material is exposed to an inoculum which is derived from compost. The composting takes place in an environment wherein temperature, aeration and humidity are closely monitored and controlled. The test method is designed to yield the percentage conversion of the carbon in the test material to evolved carbon dioxide as well as the rate of conversion.

The conditions described in this International Standard may not always correspond to the optimum conditions for the maximum degree of biodegradation to occur.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 5663:1984, *Water quality — Determination of Kjeldahl nitrogen — Method after mineralization with selenium.*

ISO 8245:1999, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).*

## 3 Definitions

For the purposes of this International Standard, the following definitions apply:

### 3.1

#### **ultimate aerobic biodegradation**

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

### 3.2

#### **composting**

an aerobic process designed to produce 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 having a limited mineral content.

### 3.3 disintegration

the physical breakdown of a material into very small fragments

### 3.4 total dry solids

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

### 3.5 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.

### 3.6 theoretical amount of evolved carbon dioxide

$\text{ThCO}_2$

the maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound

### 3.7 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

### 3.8 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

### 3.9 biodegradation phase

the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

### 3.10 plateau phase

the time, measured in days, from the end of the biodegradation phase until the end of a test

## 4 Principle

The test method determines the ultimate biodegradability and degree of disintegration of test material under conditions simulating an intensive aerobic composting process. The inoculum used consists of stabilized, mature compost derived, if possible, from composting the organic fraction of solid municipal waste.

The test material is mixed with the inoculum and introduced into a static composting vessel where it is intensively composted under optimum oxygen, temperature and moisture conditions for a test period not exceeding 6 months.

During the aerobic biodegradation of the test material, carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass) are the ultimate biodegradation products. The carbon dioxide produced is continuously monitored, or measured at regular intervals, in test and blank vessels to determine the cumulative carbon dioxide production. The percentage biodegradation is given by the ratio of the carbon dioxide produced from the test material to the maximum theoretical amount of carbon dioxide that can be produced from the test material.

The maximum theoretical amount of carbon dioxide produced is calculated from the measured total organic carbon (TOC) content. The percentage biodegradation does not include that amount of carbon converted to new cell biomass which is not metabolized in turn to carbon dioxide during the course of the test.

Additionally, the degree of disintegration of the test material is determined at the end of the test, and the loss in mass of the test material may also be determined.

## 5 Test environment

Incubation shall be in the dark or in diffused light, in an enclosure or room maintained at a constant temperature of  $58\text{ °C} \pm 2\text{ °C}$  and free from vapours inhibitory to microorganisms.

In special cases, e.g. when the melting point of the test material is low, another temperature may be chosen. This temperature shall be kept constant during the test to within  $\pm 2\text{ °C}$ . Any change in temperature shall be justified and clearly indicated in the test report.

## 6 Reagents

Use only analytical-grade reagents.

Use TLC (thin-layer chromatography) grade cellulose with a particle size of less than  $20\text{ }\mu\text{m}$  as the positive-control reference material.

## 7 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.

**7.1 Composting vessels:** Glass flasks or bottles that allow an even gas purge in an upward direction.

A minimum volume of 2 litres is required to meet the requirements specified in 8.2 and 8.3. Depending on the test material, a smaller volume may be used for screening purposes. If the loss in mass of the test material is to be determined, weigh each composting vessel empty.

**7.2 Air-supply system,** capable of supplying each composting vessel with dry or water-saturated, if required carbon-dioxide-free, air at a pre-set flow rate which shall be high enough to provide truly aerobic conditions during the test (see example given in annex A).

**7.3 Apparatus for the determination of carbon dioxide,** designed to determine carbon dioxide directly or by complete absorption in a basic solution and determination of the dissolved inorganic carbon (DIC) (see example given in annex A). If the carbon dioxide in the exhaust air 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.

**7.4 Gas-tight tubes,** to connect the composting vessels with the air supply and the carbon dioxide measurement system.

**7.5 pH meter.**

**7.6 Analytical equipment,** for the determination of dry solids (at  $105\text{ °C}$ ), volatile solids (at  $550\text{ °C}$ ) and total organic carbon (TOC), for elemental analysis of the test material and, if required, for the determination of dissolved inorganic carbon (DIC).

**7.7 Balance** (optional), to measure the mass of test vessels containing compost and test material, which is normally in the range between 3 kg and 5 kg.

**7.8 Analytical equipment** (optional), for the determination of oxygen in the air, moisture, volatile fatty acids and total nitrogen (e.g. by the Kjeldahl method as specified in ISO 5663).

## 8 Procedure

### 8.1 Preparation of the inoculum

Well aerated compost from a properly operating aerobic composting plant shall be used as the inoculum. The inoculum shall be homogeneous and free from large inert objects such as glass, stones or pieces of metal. Remove them manually and then sieve the compost on a screen of about 0,5 cm to 1 cm.

NOTE 1 It is recommended that compost from a plant composting the organic fraction of solid municipal waste be used in order to ensure sufficient diversity of microorganisms. The age of the compost should preferably be between 2 and 4 months. If such compost is not available, compost from plants treating garden or farmyard waste or mixtures of garden waste and solid municipal waste may be used.

NOTE 2 It is recommended that compost with sufficient porosity be used to enable aerobic conditions to be maintained as much as possible. Addition of structural material such as small wood particles or inert or poorly biodegradable material may prevent the compost sticking together and clogging during the test.

Determine the total dry solids and the volatile-solids content of the inoculum. The total dry solids content shall be between 50 % and 55 % of the wet solids and the volatile solids no more than about 15 % of the wet 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.

Prepare a mixture of 1 part of inoculum with 5 parts of deionized water. Mix by shaking and measure the pH immediately. It shall be between 7,0 and 9,0.

NOTE 3 For further characterization 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.

Check the activity of the inoculum during the test by means of a biodegradable reference material (see clause 6) and by measuring the carbon dioxide evolution in the blank vessels. The reference material shall be degraded by 70 % or more at the end of the test (see clause 10). 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 (see clause 10). If the production of carbon dioxide is too high, stabilize 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.

### 8.2 Preparation of test material and reference material

Determine the total organic carbon (TOC) of the test material and the reference material using e.g. ISO 8245 and report it, preferably, as grams of TOC per gram of total dry solids. Alternatively, provided the materials do not contain inorganic carbon, it is possible to determine the carbon content by elemental analysis. 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.

If the loss in mass is to be determined, determine the total dry solids and volatile solids of the test material.

NOTE The loss in mass of the test material and reference material during the test can be determined, optionally, as additional information. In the example given in annex C, the volatile-solids content of the test material is determined at the beginning of the test and compared with that at the end of the test.

Use test material in the form of granules, powder, film or simple shapes (e.g. dumb-bells). The maximum surface area of any individual piece of test material shall be about 2 cm × 2 cm. If any pieces in the original test material are larger, reduce them in size.

### 8.3 Start-up of the test

Set up at least the following numbers of composting vessels (7.1):

- a) three vessels for the test material;
- b) three vessels for the reference material;
- c) three vessels for the blank.

The amount of test mixture, containing inoculum and test material, used in the test will depend on the quality of the test material (see 8.2) 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 in each vessel. Inert material, if added (see note 2 to 8.1), is not considered in this relationship. Fill about three-quarters of the volume of the composting vessel with the test mixture. Leave sufficient headspace to allow manual shaking of the test mixture.

In a typical case, prepare composting vessels which have 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 g of dry solids and mix well. The test mixture shall have the same water content (about 50 %) as the inoculum (see 8.1). It should feel somewhat sticky and have some free water available when gently pressed by hand. 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 1 It is recommended that the ratio between organic carbon and nitrogen (C/N ratio) of the test mixture is optimized so as to ensure a good composting process. The C/N ratio for the test mixture should preferably be between 10 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, e.g. by using the Kjeldahl method as specified in ISO 5663.

Place the composting vessels in the test environment at  $58\text{ °C} \pm 2\text{ °C}$  (see clause 5) and initiate aeration using water-saturated, carbon-dioxide-free air. This can be produced by passing the air through wash-bottles filled with sodium hydroxide solution (see annex A).

NOTE 2 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).

Use a sufficiently high flow rate to ensure that aerobic conditions are maintained during the test throughout each composting vessel. Check the air flow regularly at each outlet, e.g. by using wash-bottles, to ensure that there are no leaks in any part of the system.

NOTE 3 Regular measurement 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.

Handle the reference material in the same way as the test material. The vessels for the blank contain only inoculum. It shall have the same amount of total dry solids as the vessels with test material.

#### 8.4 Incubation period

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 or an infrared analyser or, alternatively, measure the cumulative carbon dioxide evolved as dissolved inorganic carbon (DIC) after absorption in sodium hydroxide solution using e.g. ISO 8245 (see annex A). The frequency of measurement will depend on the measurement method used, the desired precision of the biodegradation curve and the biodegradability of the test mixture. If direct measurement is used, measure the carbon dioxide evolved at least twice per day at time intervals of about 6 h during the biodegradation phase and once per day later on during the plateau phase. If the cumulative method is used, measure the DIC once per day during the biodegradation phase and about twice per week during the plateau phase.

Shake the composting vessels weekly to prevent extensive channelling and to ensure uniform attack of the microorganisms on the test material.

NOTE 1 It is recommended that the air-supply system and the carbon dioxide measurement system be disconnected before shaking the compost vessels.

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 shall 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 % (see 8.1). The desired moisture content is achieved by aerating with humidified or dry air. A more drastic change in the moisture content can be obtained by adding water or by drainage via the air inlet. The weekly shaking of the compost vessels

is helpful in ensuring an even distribution of moisture. If adjustments are made, monitor the carbon dioxide evolution closely.

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, smell of the exhaust air and disintegration of the test material.

Incubate the composting vessels for a period not exceeding 6 months at a constant temperature of  $58\text{ °C} \pm 2\text{ °C}$  which is representative of full-scale composting. The incubation period can be extended until a constant plateau phase is reached, if significant biodegradation of the test material is still observable. Alternatively, the incubation period can be shortened if the plateau phase is reached earlier.

Measure the pH at regular intervals, as at the start of the test (see 8.1).

NOTE 2 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. 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, add more compost to all vessels or repeat the test using, for example, less test material or more compost.

## 8.5 Termination of the test

If the loss in mass of the test material is to be determined (see the note to 8.2), weigh the composting vessels with their test mixture. Take samples of the test mixture from all vessels. Determine the total dry solids and the volatile solids.

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 relevant physical properties, chemical analysis and photography.

## 9 Calculation and expression of results

### 9.1 Calculation of the theoretical amount of carbon dioxide

Calculate the theoretical amount of carbon dioxide  $\text{ThCO}_2$ , in grams per vessel, which can be produced by the test material using equation (1):

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

where

$M_{\text{TOT}}$  is the total dry solids, in grams, in the test material introduced into the composting vessels at the start of the test;

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

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

### 9.2 Calculation of the percentage biodegradation

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):

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

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 mean cumulative amount of carbon dioxide evolved in the blank vessels, in grams per vessel;

$\text{ThCO}_2$  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 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.

### 9.3 Calculation of loss in mass

An example of the optional calculation of loss in mass, based on the volatile-solids content, is given in annex C.

### 9.4 Expression of results

Compile tables containing the measured and calculated data on the test material, the reference material and the blanks for each day of measurement. Examples of forms for this purpose are given in annex E.

Plot the cumulative amount of carbon dioxide evolved for each composting vessel containing blank, test material and reference material as a function of time (see example given in annex B). Plot a biodegradation curve (percentage biodegradation as a function of time) for the test material and the reference material (see example in annex B). 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 the final test result.

If the test material consisted of discrete pieces, describe qualitatively the degree of disintegration of the material. Add further information such as photographs or measured values of relevant physical properties if available.

## 10 Validity of results

The test is considered as valid if

- the degree of biodegradation of the reference material is more than 70 % after 45 days;
- the difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % at the end of the test;
- 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 values) after 10 days of incubation.

## 11 Test report

The test report shall provide all pertinent information, and particularly the following:

- a reference to this International Standard;
- all information necessary to identify and describe the test material, such as dry or volatile-solids content, organic-carbon content, shape or visual appearance;
- any information necessary to identify and describe the reference material and its organic-carbon content;

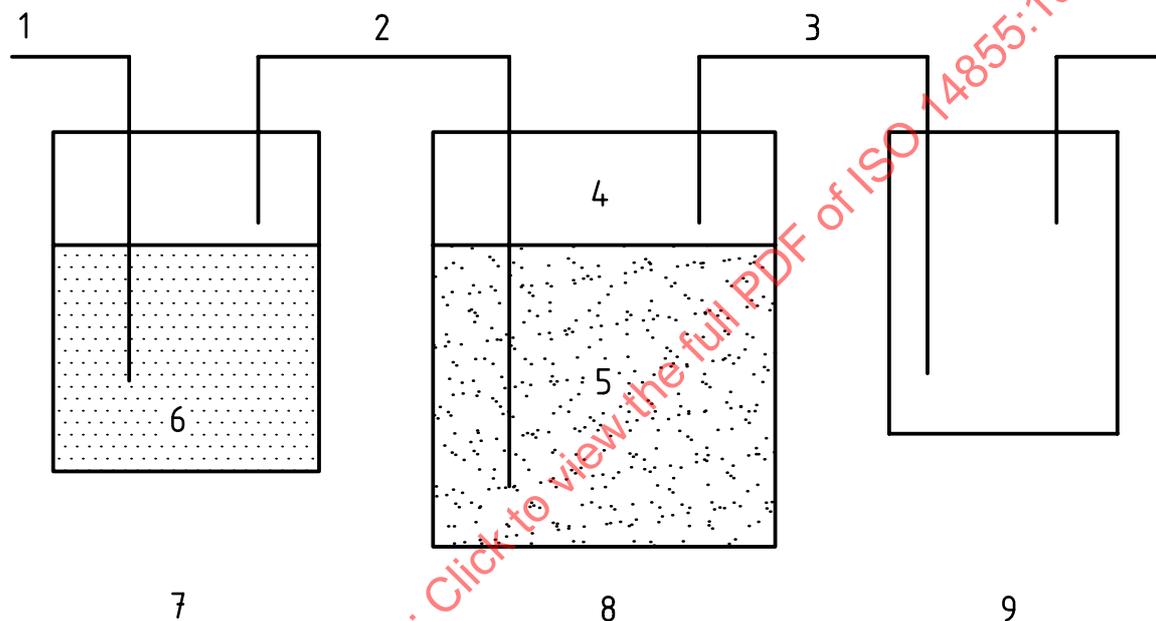
- d) the volume of the composting vessels, the amounts of inoculum, test material and reference material, and the main characteristics of the equipment used to determine the carbon dioxide and that used to determine the carbon;
- e) information on the inoculum, such as source, age, date of collection, storage, handling, stabilization, total dry solids, volatile solids, pH of suspension, total nitrogen content or volatile fatty acids, as appropriate;
- f) the results obtained for the carbon dioxide evolved and percentage biodegradation for each composting vessel and the averages, in tabular form and graphically, as well as the final degree of biodegradation of the test material and the reference material and the activity of the inoculum (CO<sub>2</sub> production after 10 days in the blank);
- g) the results of the visual observations on the inoculum and the test material during and at the end of the test, such as moisture content, fungal development, structure, colour, smell and degree of disintegration, as well as physical measurements and/or photographs;
- h) the mass of each composting vessel at the start and the end of the test, and details of any mass-loss measurements, if performed;
- i) the reasons for rejection of any test results.

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## Annex A (informative)

### 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 solution can be used to indicate the absence of carbon dioxide.



#### Key

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

**Figure A.1 — Layout of test system**

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 CO<sub>2</sub> 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 also be absorbed in a carbon dioxide trap containing e.g. a 20 g/l solution of sodium hydroxide in water and the CO<sub>2</sub> measured as dissolved inorganic carbon (DIC), e.g. in a suitable TOC analyser (using e.g. ISO 8245).

## Annex B (informative)

### Examples of graphical representation of carbon dioxide evolution and biodegradation curves

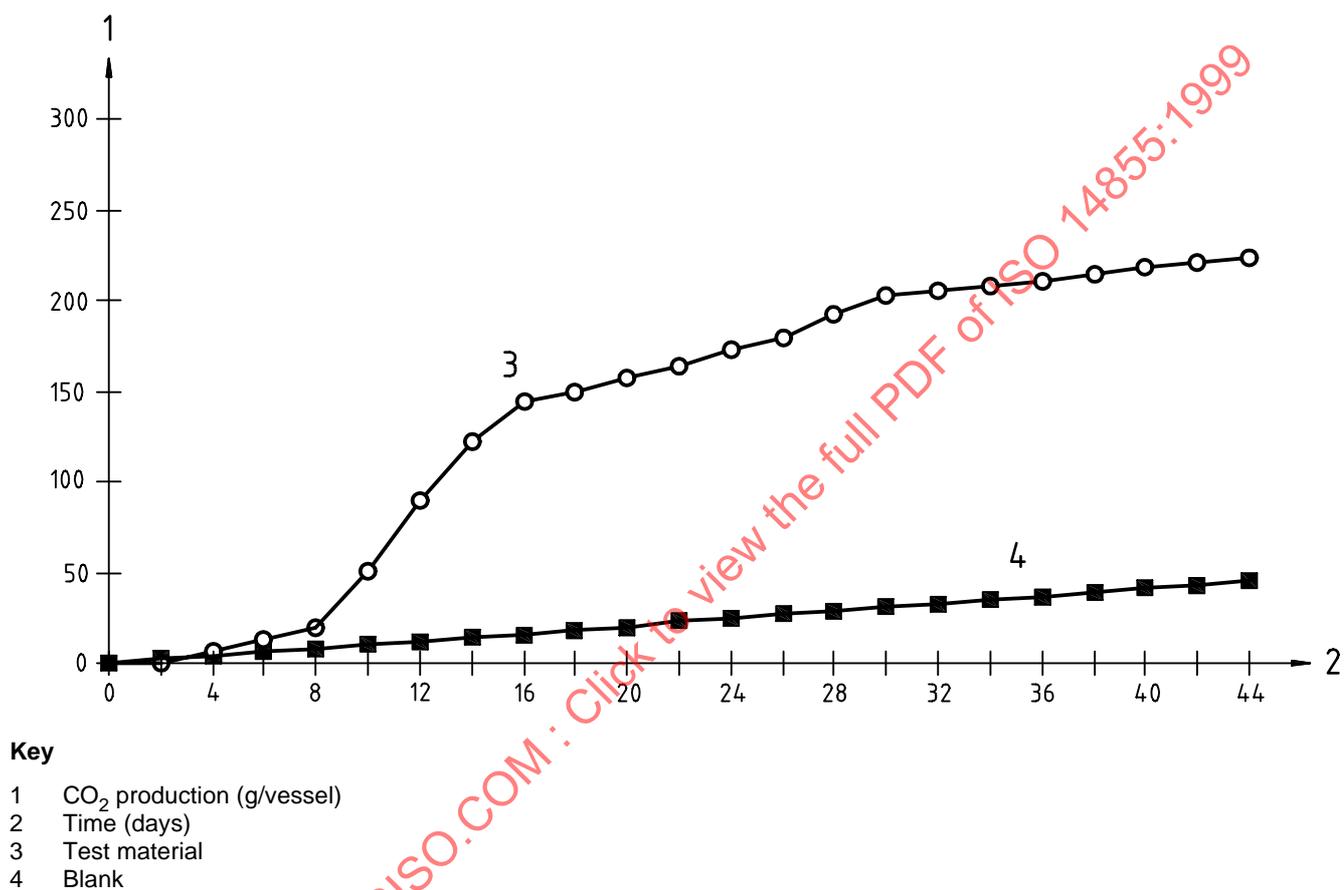
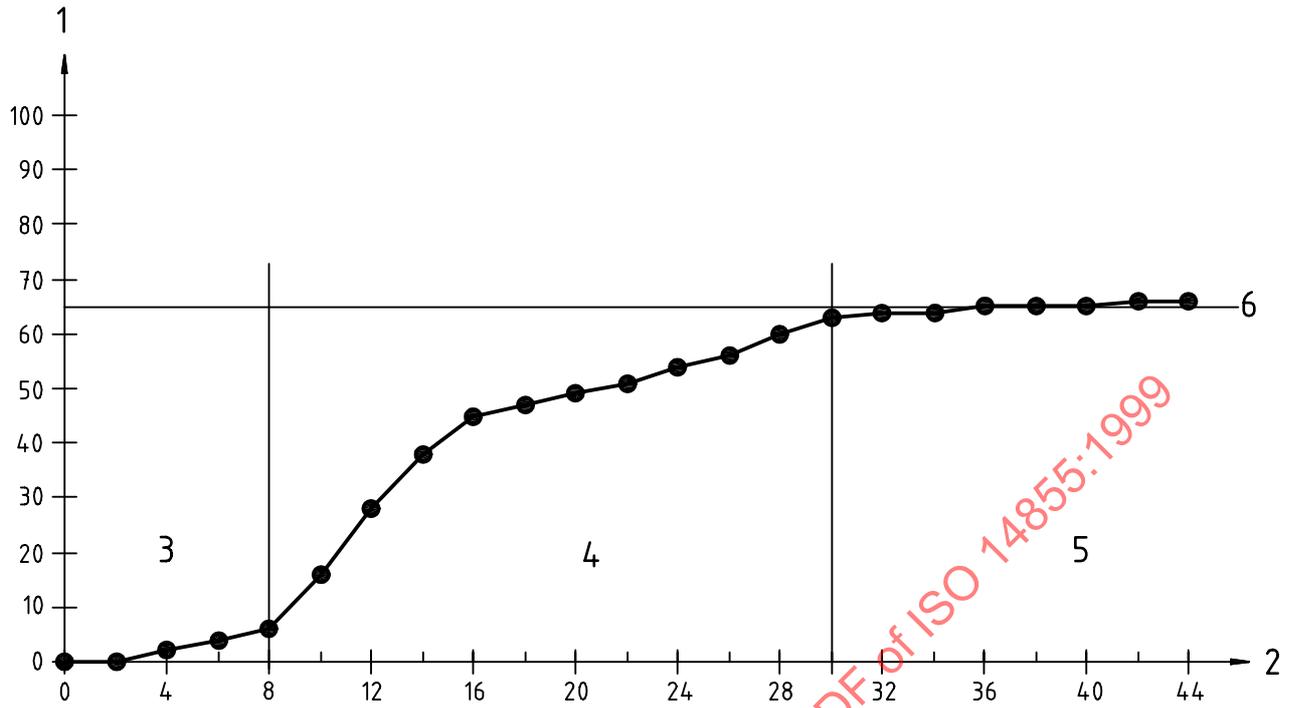


Figure B.1 — CO<sub>2</sub>-evolution curve



**Key**

- 1 Degree of biodegradation (%)
- 2 Time (days)
- 3 Lag phase
- 4 Degradation phase
- 5 Plateau phase
- 6 Mean degree of biodegradation (65 %)

**Figure B.2 — Biodegradation curve**

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## Annex C (informative)

### Example of mass-loss determination

The determination of the loss in mass of the organic matter in a test material during a composting test may provide helpful quantitative information in support of the degree of biodegradation determined primarily from measurements of CO<sub>2</sub> evolution. The following procedure gives a method of calculating this loss from measurement of the volatile solids of the test material and the inoculum compost at the beginning and the end of the test.

Abbreviations: com = inoculum compost, mat = test material, mix = mixture of test material and inoculum, ves = test vessel, wat = water.

Subscripts: w = wet material, d = total dry solids, v = volatile solids, d/w = ratio of total dry solids to wet mass, v/d = ratio of volatile solids to total dry solids, deg = degraded test material, f = test vessel, s = start of test, e = end of test, y = empty test vessel (tare), a = addition check, add = added water, B = blank (inoculum only), m = mixture of test material and inoculum, mean = mean value.

- 1 Weigh each empty test vessel to obtain the tare ( $ves_y$ ) in grams.
- 2 Determine the wet mass ( $mat_w$ ), the total dry solids ( $mat_d$ ) and the volatile solids ( $mat_v$ ) of about 10 g of the test material and calculate the ratio of total dry solids to wet mass ( $mat_{d/w}$ ) and of volatile solids to total dry solids ( $mat_{v/d}$ ).
- 3 Use the value obtained for the wet mass of the test material introduced into each test vessel at the start of the test ( $mat_{wfs}$ ) to calculate the total volatile solids ( $mat_{vfs}$ ) in each test vessel in accordance with equation (C.1), expressing the result in grams per vessel:

$$mat_{vfs} = mat_{wfs} \times mat_{d/w} \times mat_{v/d} \quad \dots (C.1)$$

- 4 Determine, before the start of the test, the wet mass ( $com_{ws}$ ), the total dry solids ( $com_{ds}$ ) and the volatile solids ( $com_{vs}$ ) of about 10 g of the compost used as the inoculum. Calculate the ratio of total dry solids to wet mass ( $com_{ds/ws}$ ) and of volatile solids to total dry solids ( $com_{vs/ds}$ ).
- 5 Use the value obtained for the wet mass of the compost introduced into each test vessel at the start of the test ( $com_{wfs}$ ) to calculate the total volatile solids in the compost ( $com_{vfs}$ ) in each vessel in accordance with equation (C.2), expressing the result in grams per vessel:

$$com_{vfs} = com_{wfs} \times com_{ds/ws} \times com_{vs/ds} \quad \dots (C.2)$$

- 6 Weigh each test vessel with the test mixture of inoculum and test material and each blank vessel containing inoculum compost only at the start ( $ves_{ms}$  and  $ves_{Bs}$ ) and the end ( $ves_{me}$  and  $ves_{Be}$ ) of the test, expressing the result in grams per vessel.
- 7 Check that the correct amounts of test material ( $mat_{wfs}$ ), inoculum ( $com_{wfs}$ ) and water ( $wat_{add}$ ) have been added to the composting vessels using equation (C.3) for the test mixtures ( $ves_{am}$ ) and equation (C.4) for the blanks ( $ves_{aB}$ ):

$$ves_{am} = ves_y + ves_{ms} = ves_y + com_{wfs} + mat_{wfs} + wat_{add} \quad \dots (C.3)$$

$$ves_{aB} = ves_y + ves_{Bs} = ves_y + com_{wfs} + wat_{add} \quad \dots (C.4)$$

- 8 For each test vessel, calculate the amount of wet mixture of test material and inoculum remaining at the end of the test ( $mix_{wfe}$ ) using equation (C.5) and for each blank calculate the amount of inoculum ( $com_{wBe}$ ) remaining using equation (C.6), expressing the results in grams per vessel:

$$\text{mix}_{\text{wfe}} = \text{ves}_{\text{me}} - \text{ves}_{\text{y}} \quad \dots \text{ (C.5)}$$

$$\text{com}_{\text{wBe}} = \text{ves}_{\text{Be}} - \text{ves}_{\text{y}} \quad \dots \text{ (C.6)}$$

- 9 Take representative samples of about 10 g of the mixture of test material and inoculum from each test vessel at the end of the test. Determine the wet mass ( $\text{mix}_{\text{we}}$ ), the total dry solids ( $\text{mix}_{\text{de}}$ ) and the volatile solids ( $\text{mix}_{\text{ve}}$ ) and calculate the ratio of total dry solids to wet mass ( $\text{mix}_{\text{de/we}}$ ) and of volatile solids to total dry solids ( $\text{mix}_{\text{ve/de}}$ ). Use the same procedure to determine the ratio of total dry solids to wet mass ( $\text{com}_{\text{de/we}}$ ) and of volatile solids to total dry solids ( $\text{com}_{\text{ve/de}}$ ) in the blanks.

- 10 Calculate the volatile solids in each test mixture at the end of the test ( $\text{mix}_{\text{vfe}}$ ) using equation (C.7) and the volatile solids in the inoculum compost in each blank vessel ( $\text{com}_{\text{vBe}}$ ) using equation (C.8), expressing the results in grams per vessel.

$$\text{mix}_{\text{vfe}} = \text{mix}_{\text{wfe}} \times \text{mix}_{\text{de/we}} \times \text{mix}_{\text{ve/de}} \quad \dots \text{ (C.7)}$$

$$\text{com}_{\text{vBe}} = \text{com}_{\text{wBe}} \times \text{com}_{\text{de/we}} \times \text{com}_{\text{ve/de}} \quad \dots \text{ (C.8)}$$

- 11 Calculate the mean value of the volatile solids in the blanks at the end of the test ( $\text{com}_{\text{vBe,mean}}$ ).

- 12 Calculate the volatile solids in the test material in each test vessel at the end of the test ( $\text{mat}_{\text{vfe}}$ ) using equation (C.9), expressing the results in grams per vessel:

$$\text{mat}_{\text{vfe}} = \text{mix}_{\text{vfe}} - \text{com}_{\text{vBe,mean}} \quad \dots \text{ (C.9)}$$

- 13 From the volatile solids, calculate the amount of degraded test material ( $\text{mat}_{\text{deg}}$ ) in each test vessel using equation (C.10), expressing the result in grams per vessel:

$$\text{mat}_{\text{deg}} = \text{mat}_{\text{vfs}} - \text{mat}_{\text{vfe}} \quad \dots \text{ (C.10)}$$

- 14 For each test vessel, calculate the percentage loss in mass of the test material, i.e. the percentage degree of biodegradation  $D_{\text{v}}$  calculated from the loss in volatile solids, using equation (C.11):

$$D_{\text{v}} = \frac{\text{mat}_{\text{deg}} \times 100}{\text{mat}_{\text{vfs}}} \quad \dots \text{ (C.11)}$$

- 15 Calculate the mean value  $D_{\text{v,mean}}$  of the degree of biodegradation.

- 16 Determine in the same way the degree of biodegradation calculated from the loss in mass of the reference material, if required.

## Annex D (informative)

### Round-robin testing

A round-robin test was carried out to validate this method. The test materials used were paper and a copolymer of poly- $\beta$ -hydroxybutyrate and poly- $\beta$ -hydroxyvalerate. Cellulose with a particle size of less than 20  $\mu\text{m}$  was used as the reference material. The test results and comments by the participants showed that the method is suitable and practicable and provides test results of high predictive value. The test results are published in

PAGGA, U., BEIMBORN, D.B., BOELENS, J., and DE WILDE, B., Determination of the Aerobic Biodegradability of Polymeric Material in a Laboratory Controlled Composting Test, *Chemosphere*, **31** (1995), pp. 4475-4487.

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