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

**Part 2:
Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test**

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

Partie 2: Mesurage gravimétrique du dioxyde de carbone libéré lors d'un essai de laboratoire



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Plastics and environment*.

This second edition cancels and replaces the first edition (ISO 14855-2:2007), which has been technically revised. It also incorporates the Technical Corrigendum ISO 14855-2:2007/Cor.1:2009.

The main changes compared to the previous edition are as follows.

- The correct values for the particle size of soda talc given in the Technical Corrigendum 1 ISO 14855-2:2007/Cor.1:2009 have been adopted.
- The following numbers of composting vessels have been provided:
 - a) three test vessels for the test mixture;
 - b) three vessels for blank controls;
 - c) three vessels for checking inoculum activity using a reference material.
- The next criterion has been added to the list of validity criteria in [Clause 10](#):
 - c) 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.

A list of all parts in the ISO 14855 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Management of plastics waste is a serious problem in the world. Plastics recovery technologies include material recovery (mechanical recycling, chemical or feedstock recycling, and biological or organic recycling) and energy recovery (heat, steam or electricity as a substitute for fossil fuels or other fuel resources). The use of biodegradable plastics is one valuable recovery option (biological or organic recycling).

Several ISO standards for determining the ultimate aerobic/anaerobic biodegradability of plastic materials have been published. In particular, ISO 14855-1 is a common test method that measures the amount of carbon dioxide evolved using methods such as continuous infrared analysis, gas chromatography or titration.

Compared with ISO 14855-1, the amounts of compost inoculum and test sample used in this document are one-tenth the size. In order to ensure the activity of the compost inoculum, inert material that gives the mixture the same texture as soil is mixed into the inoculum. The carbon dioxide evolved from the test vessel is determined by absorbing it in a carbon dioxide trap and carrying out gravimetric analysis of the absorbent. The method described in this document, which uses a closed system to capture the carbon dioxide evolved, can also be used to obtain valuable information, by means of isotopic-labelling studies, on the way in which the molecular structure of co-polymers degrades.

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Part 2:

Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test

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 document specifies a method for determining the ultimate aerobic biodegradability of plastic materials under controlled composting conditions by gravimetric measurement of the amount of carbon dioxide evolved. The method is designed to yield an optimum rate of biodegradation by adjusting the humidity, aeration and temperature of the composting vessel.

The method applies to the following materials:

- natural and/or synthetic polymers and copolymers, and mixtures of these;
- plastic materials that contain additives such as plasticizers or colorants;
- water-soluble polymers;
- materials that, under the test conditions, do not inhibit the activity of microorganisms present in the inoculum.

If the test material inhibits microorganisms in the inoculum, another type of mature compost or pre-exposure compost can be used.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11721-1, *Textiles — Determination of resistance of cellulose-containing textiles to micro-organisms — Soil burial test — Part 1: Assessment of rot-retardant finishing*

ISO 14855-1, *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions — Method by analysis of evolved carbon dioxide — Part 1: General method*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

compost

organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material and having a limited mineral content

3.2

composting

aerobic process designed to produce compost

3.3

total dry solids

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

3.4

volatile solids

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 1 to entry: The volatile-solids content is an indication of the amount of organic matter present.

3.5

ultimate aerobic biodegradation

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

3.6

theoretical amount of evolved carbon dioxide

ThCO₂

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

time from the start of a test until adaptation and/or selection of the degradation 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

Note 1 to entry: It is measured in days.

3.8

maximum level of biodegradation

degree of biodegradation of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

Note 1 to entry: It is measured as a percentage.

3.9

biodegradation phase

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

Note 1 to entry: It is measured in days.

3.10**plateau phase**

time from the end of the biodegradation phase until the end of the test

Note 1 to entry: It is measured in days.

3.11**pre-exposure**

pre-incubation of an inoculum in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the micro-organisms

3.12**pre-conditioning**

pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatization of the microorganisms to the test conditions

3.13**water-holding capacity****WHC**

mass of water that evaporates from soil saturated with water when the soil is dried to constant mass at 105 °C, divided by the dry mass of the soil

4 Principle

This method is designed to yield the optimum rate of biodegradation of a plastic material in mature compost by controlling the humidity, aeration ratio and temperature in the composting vessel. It also aims to determine the ultimate biodegradability of the test material by using a small-scale reactor. The degradation rate is periodically measured by determining the mass of the evolved carbon dioxide using an absorption column filled with soda lime and soda talc on an electronic balance.

The test material is mixed with an inoculum derived from mature compost and with an inert material such as sea sand. The sea sand plays an active part by acting as a holding body for humidity and microorganisms. Examples of suitable test arrangements are presented in [Annexes A](#) and [B](#). The amount of carbon dioxide evolved is measured at intervals on an electronic balance and the carbon dioxide content is determined using the following method. The derivation of the formula used to calculate the degree of biodegradation from the amount of carbon dioxide evolved is given in [Annex C](#). In this method, the degree of biodegradation, expressed as a percentage, is calculated by comparing the amount of carbon dioxide evolved with the theoretical amount (ThCO_2).

The test is terminated when the plateau phase of biodegradation has been attained. The standard time for termination is 45 days, but the test could be continued for up to six months.

5 Reagents

Use only analytical-grade reagents. Use only deionized water.

5.1 Soda lime, particle size between 2 mm and 4 mm, for CO_2 absorption.

5.2 Anhydrous calcium chloride, particle size between 2 mm and 3 mm, for water absorption.

5.3 Sodium hydroxide on a talc support (commonly known as soda talc), particle size between 2 mm and 3 mm, for CO_2 absorption.

5.4 Silica gel (with moisture indicator), particle size between 2 mm and 4 mm, for water absorption.

5.5 **Sea sand**, particle size between 20 mesh and 35 mesh.

5.6 **Reference material**: thin-layer chromatography (TLC) grade microcrystalline cellulose with a particle size of less than 20 µm, for use as the reference material in the positive control.

6 Apparatus

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

6.1 **Air-supply system**, capable of supplying each composting vessel with carbon-dioxide-free, water-saturated air.

The air can be prepared by supplying compressed air through a carbon dioxide trap and a humidifier (see examples in [Annexes A](#) and [B](#)), i.e. columns filled with soda lime and water, respectively. The air flow rate shall be controlled with a flow controller so that it is high enough for aerobic conditions.

6.2 **Composting vessels**

Use bottles or columns that ensure a supply of water-saturated, carbon-dioxide-free air to the contents. A suitable volume is 500 ml. If the loss in mass of the test material is to be determined, weigh each composting vessel empty before starting the test.

6.3 **System for the determination of carbon dioxide**, capable of determining carbon dioxide directly from the change in mass of a carbon dioxide trap. The carbon dioxide trap shall consist of columns filled with soda lime, soda talc and anhydrous calcium chloride. The calcium chloride should preferably be in a separate column from the soda lime and soda talc (see examples in [Annexes A](#) and [B](#)). An ammonia trap (dilute sulfuric acid) and a water trap (silica gel and calcium chloride) are required between the composting vessel and the carbon-dioxide-absorbing column.

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

6.5 **pH-meter**, used for measurement of the pH of the test mixture. It shall be accurate to 0,1 pH-units or better.

6.6 **Analytical equipment**, used for the determination of the dry solids (at 105 °C), volatile solids (at 550 °C) and total organic carbon (TOC), for elemental analysis of the test material and, if required, for the determination of dissolved inorganic carbon (DIC), volatile fatty acids, oxygen in the air, water content and total nitrogen.

6.7 **Balance**, used to periodically measure the mass of the carbon-dioxide-absorbing column, in order to determine the amount of carbon dioxide evolved, and also to measure the mass of the composting vessel containing compost and test material. A top-loading electronic balance with a display reading down to 10 mg and a capacity greater than 500 g is preferred.

6.8 **Thermostatic-control unit**, required to maintain the temperature of the composting vessels at a controlled temperature during the test (see examples given in [Annexes A](#) and [B](#)). It shall be capable of maintaining the temperature of the composting vessels constant to within ±2 °C.

6.9 **Composting bioreactor**. A box, made from polypropylene or another suitable material, having a size that allows the contents to be stirred easily with a spatula. The box shall be provided with a tightly fitting lid to avoid excessive water loss. Three holes with a diameter of about 1 cm shall be made at equal distances along the centreline of the lid. These holes allow air to enter and gases to leave the box, as well as the gradual evaporation of excess water.

7 Procedure

7.1 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 such items manually and then sieve the compost on a screen of about 3 mm mesh.

Compost can be made as follows. Wood shavings, sawdust, used mushroom beds, chaff or rice straw can be used as the carbon source. Livestock excrement is added as a source of composting microorganisms and mineral salt nutrients. This is placed in a container with a volume of about 1 m³ and mixed well. It is recommended that the compost be adjusted to a carbon/nitrogen (C/N) ratio of 15 and a carbon/phosphorous (C/P) ratio of 30. Insufficient phosphorous and nitrogen levels can be supplemented using calcium superphosphate and ammonium magnesium phosphate hexahydrate or urea respectively. Water is added to reach a water content equal to 65 %. The C/N, C/P and water-content values may also be adjusted to other values, determined by experience, depending on seasonal variations and climatic differences. The compost should be removed from the container once a week to turn it and add water if necessary, before returning it to the container to continue the composting process. The age of the compost should preferably be between two and four months.

Normally, non-exposed inoculum is preferred, especially in the case of standard tests simulating biodegradation behaviour in real composting facilities. Depending on the purpose of the test, however, pre-exposed compost may be used, provided that this is clearly stated in the test report (e.g. percent biodegradation = X %, using pre-exposed compost) and provided the method of pre-exposure is detailed in the test report.

Determine the total dry solids and volatile-solids content of the compost inoculum. The total dry solids should be between 35 % and 55 % of the wet solids and the volatile solids more than 30 % of the dry solids. Adjust the water content, if necessary, before the compost is used by adding water or drying gently, e.g. by aerating the compost with dry air.

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

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 and by measuring carbon dioxide evolution in the blank vessels. The reference material shall be degraded by 70 % or more at the end of the test. The inoculum in the blank should 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, stabilize the compost by aeration for several days before using it in a new test.

7.2 Preparation of the sea sand

Dip the sea sand in tap water. After removing floating impurities by decantation, rinse the sand sufficiently, drain off the water and dry the sand at about 105 °C.

NOTE Sea sand is an inert product that contains more than 90 % of SiO₂. It plays an important role, however, in maintaining an appropriate water content and as a support for microbial growth.

7.3 Preparation of test material and reference material

Determine the total organic carbon (TOC) of the test material and the reference material using, for example, ISO 8245 and report it preferably as grams of TOC per gram of total dry solids. Alternatively, provided that the materials do not contain inorganic carbon, it is possible to determine the carbon content by elemental analysis. For this, the test material should contain sufficient organic carbon to yield carbon dioxide in an amount suitable for determination. Normally, a minimum of 10 g of total dry solids containing 4 g of TOC is required per 500 ml-vessel.

The test material should preferably be used in powder form, but it may also be introduced as small pieces of films or as fragments of shaped articles. A maximum particle size of 250 µm in diameter is recommended.

7.4 Starting up the test

Provide at least the following numbers of composting vessels:

- a) three test vessels for the test mixture (symbol V_T);
- b) three vessels for blank controls (symbol V_B);
- c) three vessels for checking inoculum activity using a reference material (symbol V_R).

The amount of test mixture, containing inoculum and the test material, used in the test depends on the quality of the test material and the size of the composting vessels. The relation between the total dry solids of the inoculum and the total dry solids of the test material should preferably be about 6:1. If added, inert material is not considered in this relationship. The test mixture should have the same water content as the inoculum. The water content of the test mixture should be set at 80 % to 90 % of the water-holding capacity (WHC) of the test mixture. The same amount of inoculum by total dry solids should be placed in each test vessel.

In a typical case, prepare lidded vessels that have a volume of about 500 ml, weigh out, for each vessel, an amount of inoculum containing 60 g of total dry solids and add sufficient water to reach a water content of 65 %. After mixing well, leave the compost to stand at room temperature for 24 h. Then mix the compost well with sea sand with a water content of 15 % that has previously been prepared by the addition of water to about 320 g of sea sand and is used as inert material. Add 10 g, on a dry-mass basis, of test material to the mixture and mix well. It should feel like soil when handled gently. If required, measure the WHC of the test mixture in accordance with ISO 11721-1, then adjust the water content of the mixture to about 90 % of the WHC by adding water or by aerating with dry air. Introduce the mixture into the composting vessel. If vermiculite is used as the inert material, prepare it as specified in ISO 14855-1.

When mature compost preserved in the refrigerator is used as the inoculum, pre-condition the compost before using it. In a typical case, place, for each vessel, 60 g, on a total dry solids basis, of mature compost in a composting bioreactor, and adjust the water content of the compost to about 110 % of the WHC by adding water. After mixing, allow it to stand at room temperature for 24 h, and then incubate it at 58 °C for 24 h. Add the same volume of sea sand (about 320 g on a dry-mass basis) as the mature compost and mix well. Before addition, the water content of the sea sand should be adjusted to about 15 % (equal to the sea sand WHC value). The C/N ratio for the test mixture should preferably be between 10 and 40. It may be adjusted with ammonium magnesium phosphate hexahydrate or urea. Put the mixture in the composting bioreactor and incubate for a week at 58 °C. A few times per day, stir the mixture for about 10 min in order to ensure aerobic conditions and allow excess water to evaporate. After a week, adjust the water content of the mixture to about 90 % of its WHC. The final mixture should weigh about 550 g, but a different final mass could be obtained depending on the compost used (different composts will have different WHC values). Add 10 g, on a dry-mass basis, of test material to the mixture and mix well. Introduce the mixture into the composting vessels.

When ISO 14855-1 biodegradability tests are performed, mature compost with a water content of about 50 % shall be used, as specified in ISO 14855-1. Use 120 g of mature compost, containing about 60 g of total dry solids, per composting vessel. Add 10 g, on a dry-mass basis, of test material to the mature compost and mix well. Introduce the mixture into the composting vessel. If the test mixture dries out too fast, put an inert water-containing material in the vessel together with the mixture. However, the water-containing material shall not be mixed with the test mixture.

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 the Kjeldahl method described in ISO 5663.

Place the composting vessels in the test environment at $58\text{ °C} \pm 2\text{ °C}$ and initiate aeration using air that is free from carbon dioxide and has a normal water content. Both these conditions can be met by, for example, passing the air through a carbon dioxide trap filled with soda lime and a humidifier filled with water (see Annexes A and B). Adjust the air flow rate through each composting vessel to the same rate in the range 10 ml/min to 30 ml/min.

Use a sufficiently high flow rate to ensure that aerobic conditions are maintained throughout each composting vessel during the whole test. Check the air flow regularly at the outlets, e.g. by using wash-bottles or a soap bubble flow meter.

Handle the reference material in the same way as the test material. In the vessels for the blank controls, place only inoculum and sea sand, in the same amounts as in the vessels with the test material.

7.5 Measurement of the evolved carbon dioxide

Fill the ammonia-absorbing bottle with 1 M sulfuric acid to remove any ammonia from the gases which pass out of the composting vessel. Fill the two dehumidifying traps with silica gel and anhydrous calcium chloride, respectively. Fill the carbon-dioxide-absorbing column and water-absorbing column with carbon dioxide absorbent and water absorbent, respectively. The carbon dioxide absorbent should preferably be a mixture of equal quantities of soda lime and soda talc. The water absorbent should preferably be anhydrous calcium chloride. Determine the mass of this trap (i.e. both columns together) to within 10 mg on the balance. The amount of carbon dioxide evolved is determined from the increase in mass of the unit.

Change the reagents in the carbon-dioxide-absorbing and water-absorbing columns when they have reached 80 % of their absorption capacity. Note that 80 g of a mixture of equal quantities of soda lime and soda talc has the ability to absorb about 15 g of carbon dioxide.

7.6 Incubation period

Measure the amount of carbon dioxide evolved in the exhaust air from each composting vessel at intermediate time intervals by measuring the change in mass of the trap for the evolved carbon dioxide. Measure the carbon dioxide evolved at least once a day during the biodegradation phase and once every two days later on, during the plateau phase.

Stir the compost weekly to prevent extensive channelling and to ensure uniform attack by the microorganisms on the test material. Remove the compost from the vessel to do this. Add water if necessary.

Ensure that the water content 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 may also be measured using suitable instruments. In this case, the water content shall be kept at 80 % to 90 % of the WHC of the test mixture. The water content can be controlled by supplying water-saturated or dry air. The desired water content can be obtained by adding water or by drainage via the top of the composting vessel.

During the weekly stirring 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, water content, colour, fungal development, the smell of the exhaust air and the degree of disintegration of the test material.

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

During the test, if necessary, re-inoculation of the test vessels can be carried out by adding the same amount of compost to each. The origin of the compost and the date of re-inoculation shall be indicated in the test report.

Measure the pH at regular intervals, as at the start of the test.

If the pH is less than 7,0, biodegradation could be inhibited due to acidification of the compost by the rapid degradation of an easily degradable test material. In this case, measurement of the volatile fatty acid 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 shall be regarded as invalid due to acidification and the resultant inhibition of microbial activity. To prevent acidification, add more compost to all the test vessels or repeat the test using, for example, less test material, more compost or pre-exposed compost.

7.7 Termination of the test

When measuring the decrease in mass of the test material, the composting vessel containing the mixture (compost, specimen and inert material such as sea sand) can be reweighed at the end of the test. It is preferable, however, to determine the amounts of dry solids and volatile solids in each vessel after taking the mixture out of the vessel.

8 Calculation

8.1 Theoretical amount of carbon dioxide evolved by test material

The theoretical amount of carbon dioxide ($ThCO_2$) evolved by the test material in a test vessel is given, in grams, by [Formula \(1\)](#):

$$ThCO_2 = m \times w_C \times \frac{44}{12} \quad (1)$$

where

m is the mass of test material, in grams, introduced into the test vessel;

w_C is the carbon content of the test material, determined from the chemical formula or from elemental analysis, expressed as a mass fraction;

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

Calculate in the same way the theoretical amount of carbon dioxide evolved by the reference material in each vessel.

8.2 Percentage biodegradation

Calculate the percentage biodegradation D_t for each test vessel V_T from the amount of carbon dioxide evolved during each measurement interval using [Formula \(2\)](#):

$$D_t = \frac{\sum (CO_2)_T^t - \sum (CO_2)_B^t}{ThCO_2} \times 100 \quad (2)$$

where

$\sum (CO_2)_T^t$ is the cumulative amount of carbon dioxide, in grams, evolved in the test vessel V_T between the start of the test and time t ;

$\sum (CO_2)_B^t$ is the mean cumulative amount of carbon dioxide, in grams, evolved in the blank vessels V_B between the start of the test and time t (take the mean of the values obtained for the two blanks);

$ThCO_2$ is the theoretical amount of carbon dioxide, in grams, evolved by the test material.

9 Expression and interpretation 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.

Plot the cumulative amount of carbon dioxide evolved by each composting vessel containing test material, blank or reference material as a function of time. Plot biodegradation curves (percentage biodegradation as a function of time) for the test material and the reference material. Use mean values if the difference between the individual values are less than 20 %. If this is not the case, plot biodegradation curves for each composting vessel.

If a plateau phase is observed, read from the biodegradation curve the level of the plateau, i.e. the ultimate degree of biodegradation, and report it as the final test result. If no plateau phase is observed, estimate the ultimate degree of biodegradation from the cumulative amount of carbon dioxide which has evolved by the end of the test.

10 Validity of results

The test is considered as valid if:

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

If these criteria are not fulfilled, repeat the test using pre-conditioned or pre-exposed compost.

11 Test report

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

- a) a reference to this document, i.e. ISO 14855-2:2018;
- b) all information necessary to identify and describe the test material, such as dry- or volatile-solids content, organic-carbon content, shape or visual appearance;
- c) 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 placed in them, the times at which the test mixtures were stirred, and details of re-inoculation (if carried out);
- 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, and details of any pre-conditioning or pre-exposure;
- f) the results obtained for the carbon dioxide evolved, the percentage biodegradation for each composting vessel, and the averages thereof, in tabular form and graphically, as well as the ultimate degree of biodegradation of the test material and the reference material, and the activity of the inoculum (CO₂ production after 10 days in the blank);
- g) the results of visual observation of the inoculum and the test material during, and at the end of, the test, such as water content, fungal development, structure, colour and smell;
- 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 the rejection of any test results;
- j) information on the source, type and amount of inert material, such as sea sand or vermiculite, used.

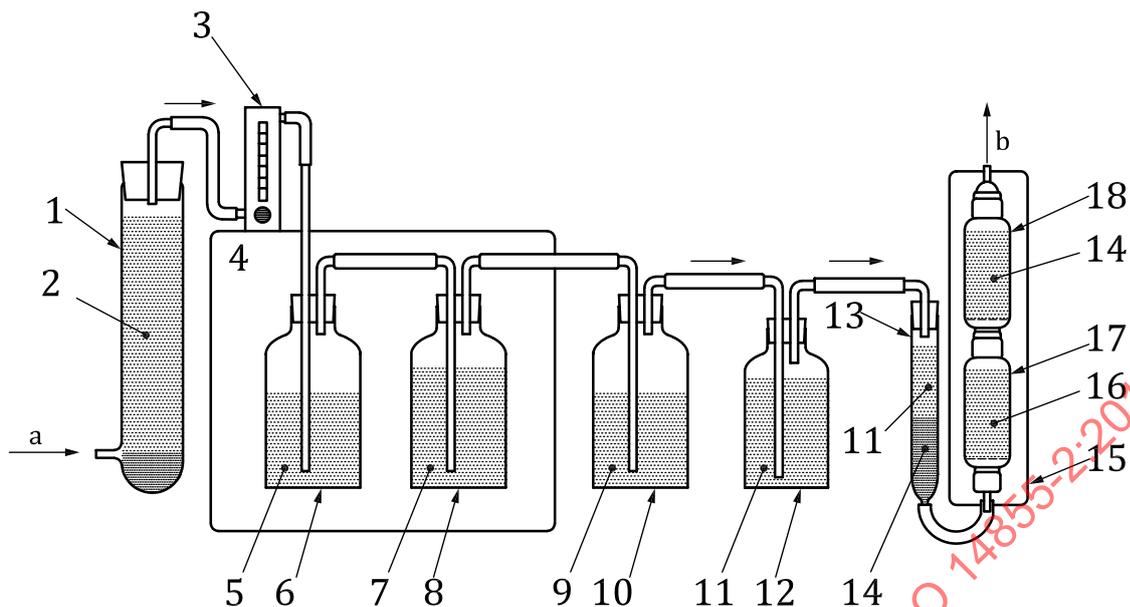
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Annex A (informative)

Basic principle of the test

A typical test set-up is shown in [Figure A.1](#). It is made up of four basic parts: (1) a composting vessel containing the mixture of test material and inoculum, located in an incubator, (2) an air-feed control system (including a trap to remove the carbon dioxide from the air, a flow-rate controller and a humidifier) to ensure accurate control of the aeration of the test mixture, (3) a gas-absorbing system for removing ammonia, hydrogen sulfide, volatile organic acids and water from the gases evolved from the composting vessel and (4) a trap which absorbs the evolved carbon dioxide for gravimetric analysis.

The composting vessel is kept at $58\text{ °C} \pm 2\text{ °C}$ in the thermostatted incubator. The mixture in each composting vessel is mixed at least once a week in another vessel. An amount of water corresponding to the loss in mass of the mixture is added and, after mixing well, the mixture is returned to the composting vessel. Carbon-dioxide-free, water-saturated air, obtained by passing the air through an absorber filled with soda lime and wash-bottles filled with water, is passed through the composting vessel at a controlled rate. Ammonia, water and any volatile organic acids in the gases evolved from the composting vessel are removed with traps containing 1 M sulfuric acid, silica gel and anhydrous calcium chloride, respectively. The carbon dioxide in these gases is trapped in the form of sodium carbonate and water (formed by the reaction between the carbon dioxide and sodium hydroxide) in a trap containing soda lime and soda talc as well as anhydrous calcium chloride. The remaining absorption capacity of the various traps can readily be monitored by observing the variation in colour of indicators or the gain in mass of the absorbent.



Key

- | | |
|---|---------------------------------------|
| 1 carbon dioxide trap | 10 ammonia trap |
| 2 soda lime | 11 silica gel |
| 3 flow meter with controller | 12 dehumidifying trap 1 |
| 4 incubator with thermostat | 13 dehumidifying trap 2 |
| 5 water | 14 anhydrous calcium chloride |
| 6 humidifier | 15 trap for evolved carbon dioxide |
| 7 mixture of compost, test material and sea sand | 16 mixture of soda lime and soda talc |
| 8 composting vessel | 17 carbon dioxide absorption column |
| 9 1 M H ₂ SO ₄ containing methyl orange indicator | 18 water absorption column |
| a Compressed air in. | |
| b Outlet. | |

Figure A.1 — Example of a test system using an incubator

Annex B (informative)

Example of an apparatus using an electrically heated composting vessel

The test set-up shown in [Figure B.1](#) operates in accordance with the same principles as that shown in [Annex A](#), but the composting vessel is designed to allow the test to be continued for longer than the normal. The main advantage of electrical heating is that it does not require the same level of maintenance as, for instance, a water bath. The fact that the composting vessel is not inside an incubator ensures easier handling of the test mixture. Dehumidifying trap 2 (see 18 in [Figure B.1](#)) and the water absorption column (see 25) have the capacity to work for about one year. The absorbents in dehumidifying trap 1 (see 17) and the carbon dioxide absorption column (see 23), however, will have to be refilled several times during the normal 45-day operation.

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