#### BS EN ISO 14855-1:2012



## **BSI Standards Publication**

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

Part 1: General method (ISO 14855-1:2012)



#### National foreword

This British Standard is the UK implementation of EN ISO 14855-1:2012. It supersedes BS EN ISO 14855-1:2007 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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EUROPEAN COMMITTEE FOR STANDARDIZATION COMITÉ EUROPÉEN DE NORMALISATION EUROPÄISCHES KOMITEE FÜR NORMUNG

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#### **Foreword**

This document (EN ISO 14855-1:2012) has been prepared by Technical Committee ISO/TC 61 "Plastics" in collaboration with Technical Committee CEN/TC 249 "Plastics" the secretariat of which is held by NBN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2013, and conflicting national standards shall be withdrawn at the latest by June 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 14855-1:2007.

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#### **Endorsement notice**

The text of ISO 14855-1:2012 has been approved by CEN as a EN ISO 14855-1:2012 without any modification.

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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.

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ISO 14855-1 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

This second edition of ISO 14855-1 cancels and replaces the first edition (ISO 14855-1:2005), of which it constitutes a minor revision intended principally to clarify the wording of the fourth paragraph in Subclause 8.1. In addition, the footnote to 6.2 concerning a possible supplier of "concrete" type vermiculite has been deleted as it appeared to be no longer valid.

This second edition also cancels and replaces the Technical Corrigendum ISO 14855-1:2005/Cor.1:2009.

ISO 14855 consists of the following parts, under the general title *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions* — *Method by analysis of evolved carbon dioxide*:

- Part 1: General method
- Part 2: Gravimetric measurement of carbon dioxide evolved in a laboratory-scale test

#### Introduction

The main method specified in this part of ISO 14855 uses a solid-phase respirometric test system based on mature compost used as a solid bed, a source of nutrients, and an inoculum rich in thermophilic microorganisms. Mature compost is a very heterogeneous and complex material. Therefore, it can be difficult to quantify the residual polymeric material left in the bed at the end of the test, to detect possible low-molecular-mass molecules released into the solid bed by the polymeric material during degradation, and to assess the biomass. As a result, it can be difficult to perform a complete carbon balance. Another difficulty which is sometimes encountered with mature compost is a "priming effect": the organic matter present in large amounts in the mature compost can undergo polymer-induced degradation, known as the "priming effect", which affects the measurement of the biodegradability.

To overcome these difficulties and to improve the reliability of the method, the mature compost can be replaced by a solid mineral medium which is used as the composting bed, thus facilitating analyses. This variant can be used to measure the biodegradation in terms of  $CO_2$  evolution, to quantify and analyse the biomass and the residues of polymeric material left in the solid bed at the end of the test, and to perform a complete carbon balance. Furthermore, the method is not significantly affected by the priming effect and can, therefore, be used to assess materials known to cause this problem with mature compost. The mineral bed can also be subjected to an ecotoxicological analysis to verify the absence of any ecotoxic activity in the bed after biodegradation.

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

#### Part 1:

#### **General method**

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 part of ISO 14855 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.

Subclauses 8.6 and 8.7 specify a variant of the method, using a mineral bed (vermiculite) inoculated with thermophilic microorganisms obtained from compost with a specific activation phase, instead of mature compost. This variant is designed to yield the percentage of carbon in the test substance converted to carbon dioxide and the rate of conversion.

The conditions described in this part of ISO 14855 may not always correspond to the optimum conditions for the maximum degree of biodegradation to occur.

#### 2 Normative references

The following referenced documents are indispensable for the application 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 5663, Water quality — Determination of Kjeldahl nitrogen — Method after mineralization with selenium

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

#### 3 Terms and definitions

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

#### 3.1

#### ultimate aerobic biodegradation

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

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

physical breakdown of a material into very small fragments

#### 3.4

#### 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

#### 3.5

#### 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 The volatile-solids content is an indication of the amount of organic matter present.

#### 3.6

#### theoretical amount of evolved carbon dioxide

#### ThCO<sub>2</sub>

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, 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

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

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

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

#### 3.11

#### activated vermiculite

vermiculite colonized by an active microbial population during a preliminary growth phase

#### 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.

Vermiculite should be used instead of mature compost

a) whenever the determination of the degree of biodegradation is affected by a priming effect induced by the test material

and/or

b) when performing a final carbon balance with biomass determination and retrieval of the residual test material.

The vermiculite bed, being inorganic, substantially reduces the priming effect, thus improving the reliability of the method. A further advantage of using vermiculite is the very small amount of carbon dioxide evolved in the blank vessels (nearly zero), because of the low level of microbial activity. This permits low levels of degradation activity to be evaluated precisely.

The mineralization rates obtained with the activated vermiculite are identical, or very similar, to those obtained with mature compost, both in terms of the final degradation level and the degradation rate.

#### 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  $^{\circ}$ C  $\pm$  2  $^{\circ}$ 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  $\pm 2$  °C. Any change in temperature shall be justified and clearly indicated in the test report.

#### 6 Reagents

#### 6.1 TLC (thin-layer chromatography) grade cellulose

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

#### 6.2 Vermiculite

Vermiculite is a clay mineral used for building purposes, known to be particularly suitable as a microbial carrier, allowing survival and full activity of microbes. The composition of the native mineral, before heat treatment, is  $Al_2O_3$  10 %, MgO 30 %, CaO 5 %,  $SiO_2$  50 % and combined  $H_2O$  5 %. When the mineral is subjected to heat treatment, it loses the combined water and expands, giving "expanded vermiculite". Expanded vermiculite in flake form shall be used. Expanded vermiculite has a large capacity for water storage, and a water content comparable with that of mature compost can be obtained in the bed.

Vermiculite can be classified into three types, as follows:

"Concrete" type: apparent density 80 kg/m $^3$  ± 16 kg/m $^3$  (at the time the material is put into sacks); particle size: 80 % between 12 mm and 4 mm, 2 % passing through a 0,5 mm sieve.

"Medium" type: apparent density 90 kg/m $^3$   $\pm$  16 kg/m $^3$ ; particle size: 80 % between 6 mm and 1 mm, 2 % passing through a 0,5 mm sieve.

"Fine" type: apparent density 100 kg/m $^3$   $\pm$  20 kg/m $^3$ ; particle size: 80 % between 3 mm and 0,7 mm, 5 % passing through a 0,5 mm sieve.

For the purposes of this part of ISO 14855, the concrete type is used.

#### 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 °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).
- **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).
- **7.9 Bioreactors for activation of the vermiculite**: Containers, with a volume between 5 I and 20 I, which are not actively aerated. The containers shall be closed in such a way as to avoid excessive drying out of the contents. Openings shall, however, be provided to allow gas exchange with the atmosphere and ensure aerobic conditions throughout the activation phase.

An example of a suitable bioreactor is a box, made of polypropylene or another suitable material, having the following dimensions:  $30 \text{ cm} \times 20 \text{ cm} \times 10 \text{ cm}$  (I, w, h). The box shall have a tightly fitting lid in order to avoid excessive loss of water vapour. In the middle of the two 20-cm-wide sides, a hole 5 mm in diameter shall be

made at a height of about 6,5 cm from the bottom of the box. It is these two holes which allow gas exchange between the atmosphere inside the box and the outside environment.

#### 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 shall be 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  $\times$  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  $^{\circ}$ C  $\pm$  2  $^{\circ}$ 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  $^{\circ}$ C  $\pm$  2  $^{\circ}$ 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 assess 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.

#### 8.6 Use of vermiculite

If using vermiculite rather than compost, the vermiculite is first activated by inoculating it with a solution containing both organic and inorganic nutrients and mature compost. The composition of the inoculum solution used shall be as given in Tables 1, 2 and 3. The ratio of vermiculite to inoculum solution shall be 1:3 (mass/volume).

Prepare the compost extract used in the inoculum solution by mixing mature compost with deionized water (20 % mass/volume) for about half an hour, then filtering the slurry with a strainer (aperture size about 1 mm). A further filtration through filter paper or centrifugation at about 1 000 rpm for 15 min can then be performed.

#### Table 1 — Composition of 1 litre of inoculum solution

| Constituent | Mineral solution (see Table 2) | Suitable nutrient broth | Urea  | Corn<br>starch | Cellulose | Compost extract |
|-------------|--------------------------------|-------------------------|-------|----------------|-----------|-----------------|
| Amount      | 500 ml                         | 13 g                    | 5,8 g | 20 g           | 20 g      | 500 ml          |

#### Table 2 — Composition of 1 litre of mineral solution

| Chemical | KH <sub>2</sub> PO <sub>4</sub> | MgSO <sub>4</sub> | CaCl <sub>2</sub><br>(10 % solution) | NaCl<br>(10 % solution) | Trace-element solution (see Table 3) |
|----------|---------------------------------|-------------------|--------------------------------------|-------------------------|--------------------------------------|
| Amount   | 1 g                             | 0,5 g             | 1 ml                                 | 1 ml                    | 1 ml                                 |

#### Table 3 — Composition of 1 litre of trace-element solution

| Chemical | H <sub>3</sub> BO <sub>3</sub> | KI     | FeCl <sub>3</sub> | MnSO <sub>4</sub> | (NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> | FeSO <sub>4</sub> |
|----------|--------------------------------|--------|-------------------|-------------------|---|-------------------|
| Amount   | 500 mg                         | 100 mg | 200 mg            | 400 mg            | 200 mg  | 400 mg            |

Mix the necessary amounts of vermiculite and inoculum solution to give a homogeneous mixture, and dispense the mixture into the bioreactors (about 1 kg of mixture in each). Weigh each bioreactor with its contents and incubate at 50  $^{\circ}$ C  $\pm$  2  $^{\circ}$ C for three/four days.

Reweigh the bioreactors daily and, if necessary, bring the mass back to its original value by adding chlorinefree tap water, deionized water or distilled water. In addition, mix the contents of each bioreactor daily with a spatula or an ordinary spoon to ensure aeration.

Vermiculite treated in this way is referred to as "activated vermiculite" and can be placed in the composting vessels for use as a solid bed instead of the mature-compost inoculum (see 8.1). For normal assessments, use 800 g of activated vermiculite in each composting vessel.

The amounts of activated vermiculite and test material used in the test will depend on the size of the composting vessels. The ratio between the dry mass of the activated vermiculite and the dry mass of the test material should preferably be about 4:1. About half of the volume of the composting vessel should be filled with the test mixture. Sufficient headspace is required to be able to manually shake the test mixture.

For normal assessments, use composting vessels which have a volume of about 3 l. Weigh out an amount of activated vermiculite corresponding to 200 g of dry solids and an amount of test material corresponding to 50 g of dry solids, and mix well before introducing the mixture into the vessels.

#### 8.7 Recovery procedure and carbon balance when using vermiculite

At the end of the test, the vermiculite beds can be extracted to recover and determine quantitatively the amount of test material remaining and the amounts of degradation by-products and/or biomass present. The bed in each composting vessel can be analysed independently or the contents of all the composting vessels in a series pooled and analysed together. The values obtained for the amount of biomass, the amount of test material remaining and the amount of by-products can be used, along with the amount of carbon evolved as  $CO_2$  during the test, to perform a final carbon balance. The amount of carbon present in the original test material is compared with the amount of carbon evolved as  $CO_2$  during the test, the amount of carbon transformed into biomass, and the amount of carbon in the remaining test material and in the degradation by-products, at the end of the test. In this way, it is possible to validate the result obtained for the degree of biodegradation.

The extractions can be performed in sequence using water and/or organic solvents, depending on the nature of the test material. For this purpose, carry out preliminary solubility trials on the test material to choose a suitable solvent.

Analytical procedures which can be used are spectroscopy (IR, UV-visible, NMR, etc.), chromatography, gravimetric analysis, elemental analysis, etc. These procedures can be applied directly to the extracts and/or to concentrates of the extracts. The extracts can also be subjected to ecotoxicological testing.

#### 9 Calculation and expression of results

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

Calculate the theoretical amount of carbon dioxide  $ThCO_2$ , in grams per vessel, which can be produced by the test material using Equation (1):

$$ThCO_2 = M_{TOT} \times C_{TOT} \times \frac{44}{12}$$
 (1)

where

 $M_{\mathsf{TOT}}$  is the total dry solids, in grams, in the test material introduced into the composting vessels

at the start of the test;

C<sub>TOT</sub> is the proportion of total organic carbon in the total dry solids in the test material, in grams

per gram;

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):

$$D_t = \frac{(\text{CO}_2)_{\text{T}} - (\text{CO}_2)_{\text{B}}}{\text{ThCO}_2} \times 100$$
 (2)

where

(CO<sub>2</sub>)<sub>T</sub> is the cumulative amount of carbon dioxide evolved in each composting vessel containing test material, in grams per vessel;

(CO<sub>2</sub>)<sub>B</sub> is the mean cumulative amount of carbon dioxide evolved in the blank vessels, in grams per vessel;

ThCO<sub>2</sub> 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

- 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.

#### 11 Test report

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

- a) a reference to this part of ISO 14855;
- 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, 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;
- j) information on the source, type and amount of vermiculite used (if applicable);
- k) if carried out, the results of the carbon balance determination.

## 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.

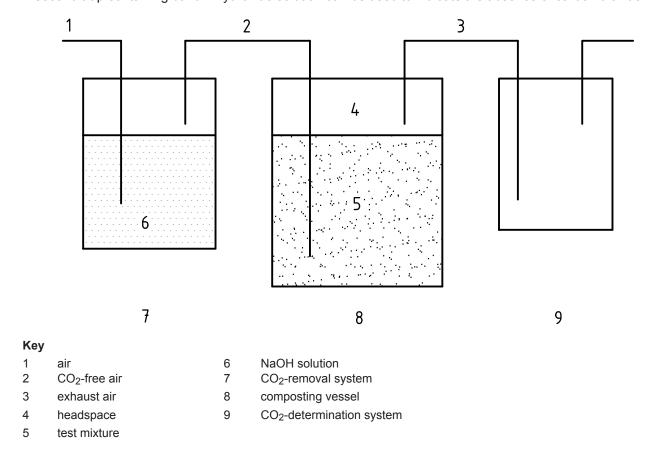


Figure A.1 — Layout of test system

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.

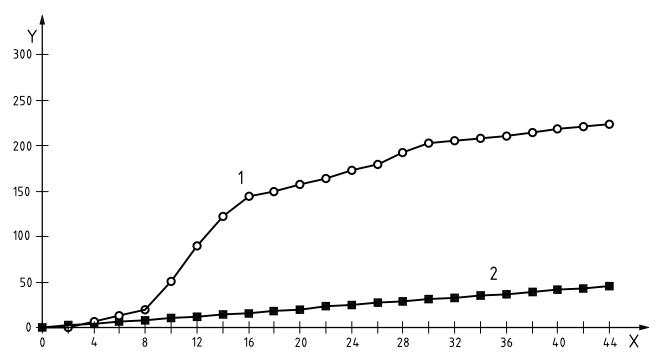
The  $CO_2$  in the exhaust air can be measured directly, e.g. with a continuous infrared analyser or a gas chromatograph. In this case, exact metering or measurement of the gas flow is necessary. Depending on the measurement instrument, it may be necessary to remove water from the air, e.g. by cooling. 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).

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## **Annex B** (informative)

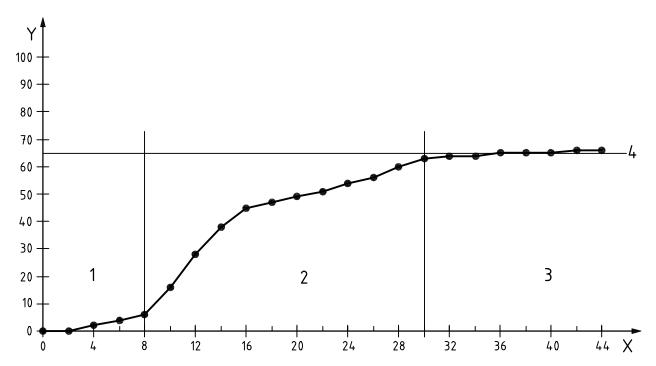
## Examples of graphical representation of carbon dioxide evolution and biodegradation curves



#### Key

- X time (days)
- Y CO<sub>2</sub> production (g/vessel)
- 1 test material
- 2 blank

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



#### Key

- X time (days)
- Y degree of biodegradation (%)
- 1 lag phase
- 2 degradation phase
- 3 plateau phase
- 4 mean degree of biodegradation (65 %)

Figure B.2 — Biodegradation curve

#### **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  $CO_2$  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.

a) Weigh each empty test vessel to obtain the tare (ves<sub>v</sub>) in grams.

- b) Determine the wet mass (mat<sub>w</sub>), the total dry solids (mat<sub>d</sub>) and the volatile solids (mat<sub>v</sub>) of about 10 g of the test material and calculate the ratio of total dry solids to wet mass (mat<sub>d/w</sub>) and of volatile solids to total dry solids (mat<sub>v/d</sub>).
- c) Use the value obtained for the wet mass of the test material introduced into each test vessel at the start of the test (mat<sub>wfs</sub>) to calculate the total volatile solids (mat<sub>vfs</sub>) in each test vessel in accordance with Equation (C.1), expressing the result in grams per vessel:

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

- d) Determine, before the start of the test, the wet mass (com<sub>ws</sub>), the total dry solids (com<sub>ds</sub>) and the volatile solids (com<sub>vs</sub>) of about 10 g of the compost used as the inoculum. Calculate the ratio of total dry solids to wet mass (com<sub>ds/ws</sub>) and of volatile solids to total dry solids (com<sub>vs/ds</sub>).
- e) Use the value obtained for the wet mass of the compost introduced into each test vessel at the start of the test (com<sub>wfs</sub>) to calculate the total volatile solids in the compost (com<sub>vfs</sub>) in each vessel in accordance with Equation (C.2), expressing the result in grams per vessel:

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

- f) 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<sub>ms</sub> and ves<sub>Bs</sub>) and the end (ves<sub>me</sub> and ves<sub>Be</sub>) of the test, expressing the result in grams per vessel.
- g) Check that the correct amounts of test material (mat<sub>wfs</sub>), inoculum (com<sub>wfs</sub>) and water (wat<sub>add</sub>) have been added to the composting vessels using Equation (C.3) for the test mixtures (ves<sub>am</sub>) and Equation (C.4) for the blanks (ves<sub>aB</sub>):

$$ves_{am} = ves_V + ves_{ms} = ves_V + com_{wfs} + mat_{wfs} + wat_{add}$$
 (C.3)

$$ves_{aB} = ves_{V} + ves_{Bs} = ves_{V} + com_{wfs} + wat_{add}$$
(C.4)

h) For each test vessel, calculate the amount of wet mixture of test material and inoculum remaining at the end of the test (mix<sub>wfe</sub>) using Equation (C.5) and for each blank calculate the amount of inoculum (com<sub>wBe</sub>) remaining using Equation (C.6), expressing the results in grams per vessel:

$$mix_{wfe} = ves_{me} - ves_{y}$$
 (C.5)

$$com_{WBe} = ves_{Be} - ves_{V}$$
 (C.6)

- i) 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 (mix<sub>we</sub>), the total dry solids (mix<sub>de</sub>) and the volatile solids (mix<sub>ve</sub>) and calculate the ratio of total dry solids to wet mass (mix<sub>de/we</sub>) and of volatile solids to total dry solids (mix<sub>ve/de</sub>). Use the same procedure to determine the ratio of total dry solids to wet mass (com<sub>de/we</sub>) and of volatile solids to total dry solids (com<sub>ve/de</sub>) in the blanks.
- j) Calculate the volatile solids in each test mixture at the end of the test (mix<sub>vfe</sub>) using Equation (C.7) and the volatile solids in the inoculum compost in each blank vessel (com<sub>vBe</sub>) using Equation (C.8), expressing the results in grams per vessel:

$$mix_{vfe} = mix_{wfe} \times mix_{de/we} \times mix_{ve/de}$$
 (C.7)

$$com_{vBe} = com_{wBe} \times com_{de/we} \times com_{ve/de}$$
 (C.8)

- Calculate the mean value of the volatile solids in the blanks at the end of the test (com<sub>vBe,mean</sub>).
- l) Calculate the volatile solids in the test material in each test vessel at the end of the test (mat<sub>vfe</sub>) using Equation (C.9), expressing the results in grams per vessel:

$$mat_{vfe} = mix_{vfe} - com_{vBe,mean}$$
 (C.9)

m) From the volatile solids, calculate the amount of degraded test material (mat<sub>deg</sub>) in each test vessel using Equation (C.10), expressing the result in grams per vessel:

$$mat_{deg} = mat_{vfs} - mat_{vfe}$$
 (C.10)

n) For each test vessel, calculate the percentage loss in mass of the test material, i.e. the percentage degree of biodegradation  $D_V$  calculated from the loss in volatile solids, using Equation (C.11):

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

- o) Calculate the mean value  $D_{v,mean}$  of the degree of biodegradation.
- p) 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 µm 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 Reference [6] (see the Bibliography).

## **Annex E** (informative)

### **Examples of forms**

#### ISO 14855-1 controlled aerobic composting test — Test report

| Test material:  | Fest material:      Reference material:                                   |   |                          |       |      |         |   |    |
|---|---|---|--------------------------|-------|------|---------|---|----|
| Origin of compost: Age of compost:  |   |   |                          |       |      |         |   |    |
| Volume of test vessels:   |   |   | CO <sub>2</sub> detern   |       |      |         |   |    |
|   |   |   |                          |       |      |         |   |    |
| Test results  |   |   |                          |       |      |         |   |    |
|   | Mean<br>biodegradation<br>calculated from<br>CO <sub>2</sub> evolved<br>% | Mean<br>biodegradation<br>calculated<br>from mass of<br>organic matter<br>% | Test<br>duration<br>days | 0     | bser | vations |   |    |
| Test material   |   |   |                          |       |      |         |   |    |
| Reference material  |   |   |                          |       |      |         |   |    |
| Validity criteria   |   |   |                          |       |      | ,       |   |    |
| Degree of biodegradation  | on of reference mate  | erial after 45 days   | > 70 %?                  |       | O Y  | es      | 0 | No |
| Difference between percentage biodegradation of reference material in the O Yes O No different vessels at end of test < 20 %? |   |   |                          |       |      |         |   |    |
| Mean CO <sub>2</sub> production in 150 mg CO <sub>2</sub> /g volatile s   |   | after 10 days in the  | e range 50 m             | ng to | O Y  | es      | 0 | No |

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## ISO 14855-1 controlled aerobic composting test — Degree of biodegradation calculated from CO<sub>2</sub> evolution

| Date | Day | (CO <sub>2</sub> ) <sub>B1</sub> | (CO <sub>2</sub> ) <sub>B2</sub> | (CO <sub>2</sub> ) <sub>B3</sub> | (CO <sub>2</sub> ) <sub>B,mean</sub> | $(CO_2)_{t1}$ | $(CO_2)_{t2}$ | $(CO_2)_{t3}$ | $D_{t1}$ | $D_{t2}$ | $D_{t3}$ | $D_{t,mean}$ |
|------|-----|----------------------------------|----------------------------------|----------------------------------|--------------------------------------|---------------|---------------|---------------|----------|----------|----------|--------------|
| Date | Day | g/vessel                         | g/vessel                         | g/vessel                         | g/vessel                             | g/vessel      | g/vessel      | g/vessel      | %        | %        | %        | %            |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |
|      |     |                                  |                                  |                                  |                                      |               |               |               |          |          |          |              |

 $(CO_2)_B = measured$  cumulative  $CO_2$  production by blank

 $(CO_2)_t$  = measured cumulative  $CO_2$  production by test material or reference material at time t

Calculations:

$$(CO_2)_{B,mean} = \frac{(CO_2)_{B1} + (CO_2)_{B2} + (CO_2)_{B3}}{3}$$

$$D_t = \frac{(\text{CO}_2)_t - (\text{CO}_2)_{\text{B,mean}}}{\text{ThCO}_2}$$

$$D_t = \frac{D_{t1} + D_{t2} + D_{t3}}{3}$$

## ISO 14855-1 controlled aerobic composting test — Degree of biodegradation calculated from loss in mass of organic matter

| Test material:      |                       | Referer               | nce material:         |                      |                      |
|---------------------|-----------------------|-----------------------|-----------------------|----------------------|----------------------|
| Test material (mat) | mat <sub>w</sub> (g): | mat <sub>d</sub> (g): | mat <sub>v</sub> (g): | mat <sub>d/w</sub> : | mat <sub>v/d</sub> : |

| Test material (mat)                   | mat <sub>w</sub> (g):  | mat <sub>d</sub> (g):  | mat <sub>v</sub> (g):  | mat <sub>d/w</sub> :   | mat <sub>v/d</sub> :   |
|---------------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Inoculum, start (com <sub>s</sub> )   | com <sub>ws</sub> (g): | com <sub>ds</sub> (g): | com <sub>vs</sub> (g): | com <sub>ds/ws</sub> : | com <sub>vs/ds</sub> : |
| Test mixture, end (mix <sub>e</sub> ) | mixwe (g):             | mix <sub>de</sub> (g): | mix <sub>ve</sub> (g): | mix <sub>de/we</sub> : | mix <sub>ve/de</sub> : |
| Inoculum, end (come)                  | comwe (g):             | com <sub>de</sub> (g): | com <sub>ve</sub> (g): | com <sub>de/we</sub> : | com <sub>ve/de</sub> : |

| Test                | mat <sub>wfs</sub> | mat <sub>vfs</sub> | wat <sub>add</sub> | ves <sub>ms</sub> | ves <sub>am</sub> | ves <sub>y</sub> | ves <sub>me</sub> | mix <sub>wfe</sub> | mix <sub>vfe</sub> | mat <sub>vfe</sub> | mat <sub>deg</sub> | $D_{V}$ |
|---------------------|--------------------|--------------------|--------------------|-------------------|-------------------|------------------|-------------------|--------------------|--------------------|--------------------|--------------------|---------|
| material            | g/<br>vessel       | g/<br>vessel       | g/<br>vessel       | g/<br>vessel      | g/<br>vessel      | g/<br>vessel     | g/<br>vessel      | g/<br>vessel       | g/<br>vessel       | g/<br>vessel       | g/<br>vessel       | %       |
| mat 1               |                    |                    |                    |                   |                   |                  |                   |                    |                    |                    |                    |         |
| mat 2               |                    |                    |                    |                   |                   |                  |                   |                    |                    |                    |                    |         |
| mat 3               |                    |                    |                    |                   |                   |                  |                   |                    |                    |                    |                    |         |
| mat <sub>mean</sub> |                    |                    |                    |                   |                   |                  |                   |                    |                    |                    |                    |         |

| Blank               | com <sub>wBs</sub> | com <sub>vBs</sub> | wat <sub>add</sub> | ves <sub>Bs</sub> | ves <sub>aB</sub> | ves <sub>y</sub> | ves <sub>Be</sub> | com <sub>wBe</sub> | com <sub>vBe</sub> |
|---------------------|--------------------|--------------------|--------------------|-------------------|-------------------|------------------|-------------------|--------------------|--------------------|
|                     | g/vessel           | g/vessel           | g/vessel           | g/vessel          | g/vessel          | g/vessel         | g/vessel          | g/vessel           | g/vessel           |
| com 1               |                    |                    |                    |                   |                   |                  |                   |                    |                    |
| com 2               |                    |                    |                    |                   |                   |                  |                   |                    |                    |
| com 3               |                    |                    |                    |                   |                   |                  |                   |                    |                    |
| com <sub>mean</sub> |                    |                    |                    |                   |                   |                  |                   |                    |                    |

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.

Calculation of degree of degradation based on volatile solids:  $D_V = \text{mat}_{\text{deg}} \times 100/\text{mat}_{\text{vfs}}$ 

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