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

Part 1: General method

Évaluation de la biodégradabilité aérobie ultime des matériaux plastiques dans des conditions contrôlées de compostage — Méthode par analyse du dioxyde de carbone libéré —
Partie 1: Méthode générale

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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

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.

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the footnote to 6.2 concerning a possible supplier of “concrete” type vermiculite has been deleted as it appeared to be no longer valid.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
$ThCO_2$
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 °C ± 2 °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 ±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 µm 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 ± 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 ± 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 l and 20 l, 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 cm × 20 cm × 10 cm (l, 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 × 2 cm. If any pieces in the original test material are larger, reduce them in size.