INTERNATIONAL STANDARD

ISO 17556

Third edition
2019-05

Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved

Plastiques — Détermination de la biodégradabilité aérobie ultime des matériaux plastiques dans le sol par mesure de la demande en oxygène dans un respiromètre ou de la teneur en dioxyde de carbone libéré

Reference number
ISO 17556:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword iv
Introduction v
1 Scope 1
2 Normative references 1
3 Terms and definitions 1
4 Principle 3
5 Test environment 4
6 Materials 4
7 Apparatus 4
8 Procedure 4
8.1 Preparation of the test material 4
8.2 Preparation of the reference material 5
8.3 Preparation of the test soil 5
8.3.1 Collection and sieving of soil 5
8.3.2 Preparation of standard soil 6
8.3.3 Measurement of soil characteristics 7
8.3.4 Adjustment of the water content and the pH of the soil 7
8.3.5 Handling and storage of the soil 7
8.4 Start-up and execution of the test 7
9 Calculation and expression of results 9
9.1 Calculation 9
9.1.1 Percentage biodegradation from oxygen consumption values 9
9.1.2 Percentage biodegradation from carbon dioxide evolved 9
9.2 Expression and interpretation of results 10
10 Validity of results 10
11 Test report 10
Annex A (informative) Principle of a manometric respirometer (example) 12
Annex B (informative) Example of a system for measuring the amount of carbon dioxide evolved 13
Annex C (informative) Examples of methods for the determination of evolved carbon dioxide 14
Annex D (informative) Theoretical oxygen demand (ThOD) 16
Annex E (informative) Example of a determination of the amount and the molecular mass of water-insoluble polymer remaining at the end of a biodegradation test 17
Annex F (informative) Examples of long-term tests 18
Annex G (informative) Interlaboratory test 22
Bibliography 26

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 on 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 the following URL: www.iso.org/iso/foreword.html.

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

This third edition cancels and replaces the second edition (ISO 17556:2012), which has been technically revised. The main changes compared to the previous edition are as follows:

a) the unit for BOD, COD and DIC has been corrected (see Clause 3);

b) the formula for calculating the percent biodegradation has been modified (see 9.1.1);

c) the test period has been revised to two years at the longest (see Clause 4);

d) the number of replicates has been corrected to three (see 9.2).

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

A number of plastic materials and products have been designed for applications ending up in or on soil. They have been developed for applications where biodegradation is beneficial from a technical, environmental, social or economic standpoint. Examples can be found in agriculture (e.g. mulching film), horticulture (e.g. twines and clips, flower pots, pins), funeral items (e.g. body bags), recreation (e.g. plastic "clay" pigeons for shooting, hunting cartridges), etc. In many cases, recovery and/or recycling of these plastic items is either difficult or not economically viable. Various types of biodegradable plastics have been developed which have been designed to biodegrade and disappear in situ at the end of their useful life. Several International Standards specify test methods for determining the ultimate aerobic or anaerobic biodegradation of plastic materials in aqueous or compost conditions. Considering the use and disposal of biodegradable plastics, it is important to establish a test method to determine the ultimate aerobic biodegradation of such plastic materials in soil.

Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved

WARNING — Appropriate precautions should be taken when handling soil because it might contain potentially pathogenic organisms. 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 in soil by measuring the oxygen demand in a closed respirometer or the amount of carbon dioxide evolved. The method is designed to yield an optimum degree of biodegradation by adjusting the humidity of the test soil.

If a non-adapted soil is used as an inoculum, the test simulates the biodegradation processes which take place in a natural environment; if a pre-exposed soil is used, the method can be used to investigate the potential biodegradability of a test material.

This method applies to the following materials:

— natural and/or synthetic polymers, copolymers or mixtures of these;

— plastic materials which contain additives such as plasticizers or colorants;

— water-soluble polymers.

It does not necessarily apply to materials which, under the test conditions, inhibit the activity of the microorganisms present in the soil. Inhibitory effects can be measured using an inhibition control or by another suitable method. If the test material inhibits the microorganisms in the soil, a lower test material concentration, another type of soil or a pre-exposed soil 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 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

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 http://www.electropedia.org/

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
biochemical oxygen demand
BOD
mass concentration of dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter

Note 1 to entry: It is expressed as milligrams of oxygen uptake per kilogram of test soil.

3.3
dissolved organic carbon
DOC
part of the organic carbon in water which cannot be removed by specified phase separation

Note 1 to entry: It is expressed as milligrams of carbon per litre.

Note 2 to entry: Typical means of separation are centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or membrane filtration using membranes with pores of diameter 0,2 µm to 0,45 µm.

3.4
theoretical oxygen demand
ThOD
maximum theoretical amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

Note 1 to entry: It is expressed as milligrams of oxygen uptake per milligram or gram of test compound.

3.5
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

Note 1 to entry: It is expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.

3.6
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)

3.7
biodegradation phase
time, measured in days, from the end of the *lag phase* (3.6) of a test until about 90 % of the *maximum level of biodegradation* (3.8) has been reached

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

3.9
plateau phase
time from the end of the *biodegradation phase* (3.7) until the end of the test

Note 1 to entry: It is measured in days.

3.10
pre-conditioning
pre-incubation of soil 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 performance of the test by acclimatization of the microorganisms to the test conditions

3.11
pre-exposure
pre-incubation of soil in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the soil to biodegrade the test material by adaptation and/or selection of the microorganisms

3.12
water content
mass of water which evaporates from the soil when the soil is dried to constant mass at 105 °C, divided by the dry mass of the soil

Note 1 to entry: This is simply the ratio between the mass of the water and that of the soil particles in a soil sample.

3.13
total water-holding capacity
mass of water which 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

3.14
total organic carbon
TOC
amount of carbon bound in an organic compound

Note 1 to entry: It is expressed as milligrams of carbon per 100 mg of the compound.

4 Principle

This method is designed to yield the optimum rate of biodegradation of a plastic material in a test soil by controlling the humidity of the soil, and to determine the ultimate biodegradability of the material.

The plastic material, which is the sole source of carbon and energy, is mixed with the soil. The mixture is allowed to stand in a flask over a period of time during which the amount of oxygen consumed (BOD) or the amount of carbon dioxide evolved is determined. Provided the CO_2 evolved is absorbed, the BOD can be determined, for example, by measuring the amount of oxygen required to maintain a constant gas volume in a respirometer flask, or by measuring either automatically or manually the change in volume or pressure (or a combination of the two). An example of a suitable respirometer is shown in Annex A. The amount of carbon dioxide evolved is measured at intervals dependent on the biodegradation kinetics of the test substance by passing carbon-dioxide-free air over the soil and then determining the carbon dioxide content of the air by a suitable method. Examples of suitable methods are given in Annexes B and C.

The level of biodegradation, expressed as a percentage, is determined by comparing the BOD with the theoretical oxygen demand (ThOD) or by comparing the amount of carbon dioxide evolved with the theoretical amount ($ThCO_2$). The influence of possible nitrification processes on the BOD has to be considered. The normal test period is six months. The test may be shortened or extended until the plateau phase (see 3.9) is reached, but the total test period shall not exceed two years.

Unlike ISO 11266, which is used for a variety of organic compounds, this document is specially designed to determine the biodegradability of plastic materials.

5 Test environment

Incubation shall take place in the dark or in diffused light in an enclosure which is free from vapours toxic to microorganisms and is maintained at a temperature constant to within ±2 °C in the range between 20 °C and 28 °C, preferably 25 °C.

6 Materials

6.1 Distilled water, containing less than 2 mg of DOC per litre.

6.2 Carbon dioxide absorber, preferably soda lime pellets.

7 Apparatus

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

7.1 Closed respirometer, including test flasks and all other necessary equipment, located in a constant-temperature enclosure or in a thermostatically controlled apparatus (e.g. a water-bath). An example is described in Annex A.

Any respirometer capable of determining with sufficient accuracy the biochemical oxygen demand is suitable, preferably an apparatus which measures and automatically replaces the oxygen consumed so that no oxygen deficiency and no inhibition of the microbial activity occurs during the degradation process.

7.2 Apparatus for measuring the amount of carbon dioxide evolved

7.2.1 Test flasks: glass vessels (e.g. conical flasks or bottles), fitted with tubing impermeable to carbon dioxide to allow purging with gas, and located in a constant-temperature enclosure or in a thermostatically controlled apparatus (e.g. a water-bath).

7.2.2 CO_2-free-air production system, capable of supplying CO_2-free air at a flow rate of several ml/min to each test flask, held constant to within ±10 % (see example of system, including test vessels, in Annex B). Alternatively, the incubation apparatus shown in ASTM D5988 may be used.

7.2.3 Analytical equipment for accurately determining carbon dioxide. Typical examples are a carbon dioxide IR analyser, a dissolved inorganic carbon (DIC) analyser, apparatus for titrimetric determination after complete absorption in a basic solution (see Annex C), and apparatus for the gravimetric determination of carbon dioxide in accordance with ISO 14855-2.

7.3 Analytical balance.

7.4 pH-meter.

8 Procedure

8.1 Preparation of the test material

The test material shall be of known mass and contain sufficient carbon to yield a BOD or a quantity of carbon dioxide that can be adequately measured by the analytical equipment used. Calculate the TOC

from the chemical formula or determine it by a suitable analytical technique (e.g. elemental analysis or measurement in accordance with ISO 8245) and calculate the ThOD or $ThCO_2$ (see Annexes C and D).

NOTE Although elemental analysis is generally less accurate for macromolecules than for low-molecular-mass compounds, the accuracy is usually acceptable for the purposes of calculating the ThOD or $ThCO_2$.

The amount of test material shall be sufficient to outweigh any variations in the background oxygen consumption or any carbon dioxide evolved from the test soil: 100 mg to 300 mg of test material to 100 g to 300 g of soil is usually adequate. The maximum amount of test material is limited by the oxygen supply to the test system. The use of 200 mg of test material with 200 g of soil is recommended unless the soil contains an excessively large amount of organic matter.

When using test systems based on the determination of the carbon dioxide evolved, higher test material amounts can be used (e.g. 2 500 mg for 200 g of soil) in order to increase the difference between the test material CO_2 production and the blank control CO_2 production. Furthermore, a greater amount of test material will be required if a final mass balance determination is to be carried out (see Annex E).

Pre-aeration of the test material or the addition of inert material is recommended, if necessary, to reduce the respiration of the soil in the blank flasks.

The test material should preferably be used in powder form, but it may also be introduced in the form of films, fragments or shaped articles.

Test samples may be reduced in size by means of cryogenic milling.

Experiments have shown that the ultimate degree of biodegradation is almost independent of the form and shape of the test material. The speed of biodegradation, however, depends on the form and shape of the material. Test materials of similar form and shape should therefore be used if different kinds of plastic material are to be compared in tests of the same duration. If the test material is in the form of a powder, small particles of known size distribution should be used. A particle-size distribution with its maximum at 250 µm diameter is recommended. If the test material is not in powder form, the size of the pieces of material should not be greater than 5 mm × 5 mm. Also, the size of the test equipment used might depend on the form of the test material. It should be ascertained that no undesired changes are caused in the test material due to the design of the equipment, such as grinders, used. Normally, processing of the test material will not significantly influence the degradation behaviour of the material (e.g. the use of powder in the case of composites).

Optionally, determine the hydrogen, oxygen, nitrogen, phosphorus and sulfur contents, as well as the molecular mass of the test material, using, for example, size exclusion chromatography. Preferably, plastic materials without additives such as plasticizers should be tested. When the material does contain such additives, information on their biodegradability will be needed to assess the biodegradability of the polymeric material itself.

For details on how to handle compounds with limited solubility in water, see ISO 10634.

8.2 Preparation of the reference material

Use as reference material a well-defined biodegradable polymer {microcrystalline-cellulose powder, ashless cellulose filters or poly-(R)-3-hydroxybutyrate [(R)-PHB]}. If possible, the physical form and size of the reference material should be comparable to that of the test material.

As a negative control, a non-biodegradable polymer (e.g. polyethylene) in the same physical form as the test material may be used.

8.3 Preparation of the test soil

8.3.1 Collection and sieving of soil

Use natural soil collected from the surface layer of fields and/or forests. If the potential biodegradability of the test material is to be assessed, this soil may be pre-exposed to the test material. Sieve the soil to

give particles of less than 5 mm, preferably less than 2 mm, in size and remove obvious plant material, stones and other inert materials.

It is important to remove organic solids, such as straw, as far as practicable because they can decompose during the test and influence the results.

The soil may be pre-conditioned but, normally, pre-exposed soil should not be used, especially when biodegradation behaviour in natural environments is being simulated. Depending on the purpose of the test, however, pre-exposed soil may be used, provided that this is clearly stated in the test report (e.g. percent biodegradation = *x* %, using pre-exposed soil) and the method of pre-exposure detailed. Pre-exposed soil can be obtained from suitable laboratory biodegradation tests conducted under a variety of conditions or from samples collected from locations where relevant environmental conditions exist (e.g. contaminated areas or industrial treatment plants).

Record the sampling site, its location, the presence of plants or previous crops, the sampling date, the sampling depth and, if possible, the soil history, such as details of fertilizer and pesticide application.

8.3.2 Preparation of standard soil

As an alternative to the natural soil described in 8.3.1, a standard soil may be used. The composition of the standard soil is shown in Table 1. The use of standard soil is very useful in determining the biodegradability of plastic materials in bulky soils (loamy or clayey soils), reducing handling and aeration problems.

Table 1 — Standard-soil composition

Constituent	Remarks	Dry mass, g/kg
Industrial quartz sand	Predominantly fine sand in which the size of more than 50 % of the particles lies in the range 0,05 mm to 0,2 mm	700 g/kg
Clay	Kaolinite clay (containing not less than 30 % kaolinite) or calcium bentonite	100 g/kg
Natural soil	See 8.3.1	160 g/kg
Mature compost	Use well-aerated compost from an aerobic composting plant. In order to stabilize the microbial activity in the standard soil, it is recommended that one-year-matured compost be used. If this is not possible, use a compost which has matured for a minimum of two-three months. The compost shall be homogeneous and free from large, inert objects, such as pieces of glass, stones or pieces of metal. Remove them manually and then sieve the compost through a screen of mesh size about 2 cm to 5 cm.	40 g/kg

To the soil specified in Table 1 are added the salts listed in Table 2, preferably dissolved in water and preferably at the moment of adjustment of the water content (see 8.3.4).

Table 2 — Added salts

Constituent	Molecular formula	g/kg of soil
Potassium dihydrogenphosphate	KH_2PO_4	0,2
Magnesium sulfate	$MgSO_4$	0,1
Sodium nitrate	$NaNO_3$	0,4
Urea	$CO(NH_2)_2$	0,2
Ammonium chloride	NH_4Cl	0,4

A round-robin test was carried out to validate the standard soil (see Annex G).