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

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



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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 17556 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

## Introduction

With the increasing use of plastics, their recycling and disposal have become a major issue. As a first priority, recycling needs to be promoted. Complete recycling of plastics, however, is difficult. For example, plastic litter, which comes mainly from consumers, is difficult to recycle completely. Other examples of plastic materials which are difficult to recycle are fishing tackle, agricultural mulch films and water-soluble polymers. These materials tend to "leak" from closed waste-management infrastructures into the natural environment. Biodegradable plastics are now emerging as one of the options available to solve such environmental issues. Several International Standards specifying methods for determining the ultimate aerobic/anaerobic biodegradability of plastic materials in aqueous/compost conditions have been published. In view of the use and disposal of biodegradable plastics, it is therefore very important to establish a method of determining the ultimate aerobic biodegradability of such materials in soil.

# Plastics — Determination of the ultimate aerobic biodegradability 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 may contain potentially pathogenic organisms. Toxic test compounds and those whose properties are unknown should be handled with care.**

## 1 Scope

This International Standard 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 soil 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.

— Materials which, under the test conditions, do not inhibit the activity of the microorganisms present in the soil. Inhibitory effects can be measured using an inhibition control or by another suitable method (see e.g. ISO 8192). 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 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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10634, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium*

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.

**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 in water, expressed as milligrams of oxygen uptake per milligram or gram of test compound

**3.3**
**dissolved organic carbon**
**DOC**
that part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 μm to 0,45 μm diameter



**3.4**
**theoretical oxygen demand**
**ThOD**
maximum theoretical amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula; expressed as milligrams of oxygen uptake per milligram or gram of test compound



**3.5**
**theoretical amount of carbon dioxide evolved**
**$ThCO_2$**
maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula; 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

**3.7**
**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

**3.8**
**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**
**plateau phase**
time, measured in days, from the end of the biodegradation phase until the end of the test

**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 (i.e. the ratio between the mass of the water and that of the soil particles in a soil sample)

**3.13**
**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

## 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. The BOD is 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 in per cent, 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 test is terminated when a constant level of biodegradation has been attained or, at the latest, after six months.

Unlike ISO 11266, which is used for a variety of organic compounds, this International Standard 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 ± 1 °C, preferably between 20 °C and 25 °C, but other temperatures may be used for particular test environments.

## 6 Materials

**6.1 Distilled water**, containing less than 2 mg/l of DOC.

**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 thermostatted apparatus (e.g. water-bath). For an example, see Annex A.

NOTE 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 determining 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 thermostatted apparatus (e.g. 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 $\pm$ 10 % (see example of system, including test vessels, in Annex B). Alternatively, the incubation apparatus shown in ASTM D 5988 may be used.

**7.2.3 Analytical instrument for determining carbon dioxide**, consisting of any suitable apparatus with sufficient accuracy, e.g. a carbon dioxide or DIC analyser or apparatus for titrimetric determination after complete absorption in a basic solution (see examples in Annex C).

**7.3 Analytical balance**.

**7.4 pH-meter**.



## 8 Procedure



### 8.1 Preparation of 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 1 Although elemental analysis is generally less precise for macromolecules than for low-molecular-mass compounds, the precision 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.

NOTE 2 Pre-aeration of the test material or the addition of inert material is recommended, as and when necessary, to reduce the influence on respiration of the soil in 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.

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, does depend 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 length. 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 may depend on the form of the test material. It should be ascertained that no substantial mechanical aberrations occur due to the design of the equipment. 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 poorly water-soluble compounds, see ISO 10634.

## 8.2 Preparation of reference material

Use as reference material a well-defined biodegradable polymer (for example, microcrystalline cellulose powder, ashless cellulose filters or poly-$\beta$-hydroxybutyrate) with a biodegradability similar to that of the test material. If possible, the 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 form as the test material can 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, or a soil which has been pre-exposed to the test material. Sieve the soil to give particles of less than 2 mm in size and remove obvious plant material, stones and other inert materials.



NOTE 1 It is important to remove organic solids, such as straw, as far as practicable because they can decompose during the test.

NOTE 2 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. per cent biodegradation $= x$ %, using pre-exposed soil) and the method of pre-exposure detailed in the test report. 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 history such as details of fertilizer and pesticide application.

### 8.3.2 Measurement of soil characteristics

Knowledge of the soil characteristics is essential for full interpretation of the results of the study. It is therefore recommended that at least the following tests be performed on the soil selected:

a) **total water-holding capacity,** in accordance with ISO 11274;

b) **pH of the soil,** in accordance with ISO 10390;

c) **organic-matter content,** in accordance with ISO 10694.

### 8.3.3 Adjustment of the water content and the pH of the soil

Adjust the water content of the soil to a suitable value for the test material by adding an appropriate amount of water to the soil, or by drying the soil in the air in a shaded place followed by addition of an appropriate amount of water. Adjust the pH of the soil to between 6,0 and 8,0 if it is not already within this range.