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## Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide

*Évaluation de la biodégradabilité aérobie ultime des matériaux plastiques en milieu aqueux — Méthode par analyse du 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.

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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document can 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](http://www.iso.org/patents)).

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

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 249, *Plastics*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This third edition cancels and replaces the second edition (ISO 14852:2018), which has been technically revised. The main changes compared to the previous edition are as follows:

- in the Scope and [Clause 8](#), soil and compost have been excluded for the inoculums used in this document;
- in [8.4](#), number of flasks for checking the inoculum activity have been changed from three to two;
- the validity criteria has been revised to conform with ISO 14851.

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](http://www.iso.org/members.html).

## Introduction

With the increasing use of plastics, their recovery and disposal have become a major issue. As a first priority, recovery should be promoted. Biodegradable plastics are now emerging as one of the options available to solve such environmental problems. Plastic materials, such as products or packaging, which are sent to composting facilities should be potentially biodegradable. Therefore, it is very important to determine the potential biodegradability of such materials and to obtain an indication of their potential biodegradability.

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# Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide

**WARNING** — Sewage, activated sludge can contain potentially pathogenic organisms. Therefore, appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

## 1 Scope

This document specifies a method, by measuring the amount of carbon dioxide evolved, for the determination of the degree of aerobic biodegradability of plastic materials, including those containing formulation additives. The test material is exposed in a synthetic medium under standardized laboratory conditions to an inoculum from activated sludge under aerobic conditions.

The conditions used in this document do not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but this test method is designed to measure the biodegradation of plastic materials and give an indication of their potential biodegradability.

The method enables the assessment of the biodegradation to be improved by calculating a carbon balance (optional, see [Annex C](#)).

The method applies to the following materials:

- natural and/or synthetic polymers, copolymers or mixtures thereof;
- plastic materials which contain additives such as plasticizers, colorants or other compounds;
- water-soluble polymers;
- materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see, for example, ISO 8192<sup>[1]</sup>). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum 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 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.

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

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

**3.1 ultimate aerobic biodegradability**  
breakdown ratio by expressed as percentage 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 activated sludge**  
mixture of organic materials and biomass produced in the aerobic treatment of waste water by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

**3.3 concentration of suspended solids**  
amount of solids obtained by filtration or centrifugation of a known volume of *activated sludge* (3.2) and drying at about 105 °C to constant mass

**3.4 dissolved inorganic carbon**  
**DIC**  
part of the inorganic carbon in water which cannot be removed by specified phase separation

Note 1 to entry: Phase separation can be achieved for example by centrifugation at 40 000 m·s<sup>-2</sup> for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

**3.5 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

Note 1 to entry: It is calculated from the molecular formula.

Note 2 to entry: It is expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.

**3.6 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.

[SOURCE: ISO 17556:2012, 3.14]

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

Note 1 to entry: Phase separation can be achieved for example by centrifugation at 40 000 m·s<sup>-2</sup> for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

**3.8 lag phase**  
time 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* (3.9)

Note 1 to entry: It is measured in days.



**3.9****maximum level of biodegradation**

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

Note 1 to entry: It is measured in per cent.

**3.10****biodegradation phase**

time from the end of the *lag phase* (3.8) of a test until the *plateau phase* (3.11) has been reached

Note 1 to entry: It is measured in days.

**3.11****plateau phase**

time from the end of the *biodegradation phase* (3.10) until the end of a test

Note 1 to entry: It is measured in days.

**3.12****pre-exposure**

pre-incubation of an *inoculum* (3.14) in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

**3.13****pre-conditioned**

pre-incubated *inoculum* (3.14) under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatization of the microorganisms to the test conditions

**3.14****inoculum**

microorganisms or other material used in an *inoculation* (3.15)

Note 1 to entry: Also called inoculant.

**3.15****inoculation**

introduction of microorganisms into a culture medium in order to start a biological process

**4 Principle**

The biodegradability of a plastic material is determined using aerobic, mesophilic microorganisms in an aqueous system. The test mixture contains an inorganic medium, the organic test material (the sole source of carbon and energy) with a concentration between 100 mg/l and 2 000 mg/l of organic carbon and activated sludge as the inoculum. If higher concentrations of test material are used, then an optimised test medium should be applied.

NOTE Lower concentrations such as those between 20 mg/l and 40 mg/l of organic carbon have been tested and found suitable.

The mixture is agitated in test flasks and aerated with carbon-dioxide-free air over a period of time depending on the biodegradation kinetics, but not exceeding 2 months. The carbon dioxide evolved during the microbial degradation is determined by a suitable analytical method, examples of which are given in [Annexes A](#) and [B](#).

The level of biodegradation is determined by comparing the amount of carbon dioxide evolved with the theoretical amount ( $\text{ThCO}_2$ ) and expressed in per cent. The test result is the maximum level of biodegradation, determined from the plateau phase of the biodegradation curve. Optionally, a carbon balance can be calculated to give additional information on the biodegradation (see [Annex C](#)).

Unlike ISO 9439, which is used for a variety of organic compounds, this document is specially designed for the determination of the biodegradation of plastic materials. The special requirements necessary affect the choice of the inoculum and the test medium, and there is the possibility of improving the evaluation of the biodegradability by calculating a carbon balance.

## 5 Test environment

Incubation shall take place in the dark or in diffuse light in an enclosure which is free from vapours inhibitory to microorganisms and which is maintained at a constant temperature, preferably between 20 °C and 25 °C, to an accuracy of  $\pm 1$  °C.

## 6 Reagents

Use only reagents of recognized analytical grade.

**6.1 Distilled or deionized water**, free of toxic substances (copper in particular) and containing less than 2 mg/l of DOC.

### 6.2 Test medium.

Depending on the purpose of the test, different test media can be used. For example, if a test material is used at higher concentrations, use the optimized test medium (6.2.2) with higher buffering capacity and nutrient concentrations.

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#### 6.2.1 Standard test medium.

##### 6.2.1.1 Solution A.

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Dissolve the following in water (6.1) and make up to 1 000 ml.

anhydrous potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	8,5 g
anhydrous dipotassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ )	21,75 g
disodium hydrogen phosphate dihydrate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ )	33,4 g
ammonium chloride ( $\text{NH}_4\text{Cl}$ )	0,5 g

The correct composition of the solution can be checked by measuring the pH, which should be 7,4.

##### 6.2.1.2 Solution B.

Dissolve 22,5 g of magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) in water (6.1) and make up to 1 000 ml.

##### 6.2.1.3 Solution C.

Dissolve 36,4 g of calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) in water (6.1) and make up to 1 000 ml.

##### 6.2.1.4 Solution D.

Dissolve 0,25 g of iron(III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) in water (6.1) and make up to 1 000 ml.

Prepare this solution freshly before use to avoid precipitation, or add a drop of concentrated hydrochloric acid (HCl) or a drop of 0,4 g/l aqueous solution of ethylenediaminetetraacetic acid (EDTA).

**6.2.1.5 Preparation.**

To prepare 1 l of test medium, add the following, to about 500 ml of water (6.1):

- 10 ml of solution A (6.2.1.1);
- 1 ml of each of solutions B (6.2.1.2), C (6.2.1.3), D (6.2.1.4).

Make up to 1 000 ml with water (6.1).

Prepare the test medium freshly before use. The solutions A up to C can be stored up to 6 months in the dark at room temperature. The same applies for solution D in case HCl or EDTA has been added.

**6.2.2 Optimized test medium.**

This optimized medium is highly buffered and contains more inorganic nutrients. This is necessary to keep the pH constant in the system during the test, even at high concentrations of the test material. The medium contains about 2 400 mg/l of phosphorus and 50 mg/l of nitrogen and is therefore suitable for concentrations in the test material of up to 2 000 mg/l of organic carbon. If higher or lower test-material concentrations are used, increase or decrease respectively the nitrogen content to keep the C:N ratio at about 40:1.

**6.2.2.1 Solution E.**

Dissolve the following in water (6.1) and make up to 1 000 ml.

anhydrous potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	37,5 g
disodium hydrogen phosphate dihydrate ( $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ )	87,3 g
ammonium chloride ( $\text{NH}_4\text{Cl}$ )	2,0 g

**6.2.2.2 Solution F (trace-element solution, optional).**

Dissolve in 10 ml of aqueous HCl solution (25 %, 7,7 mol/l), in the following sequence:

- a) 70 mg of  $\text{ZnCl}_2$ ;
- b) 100 mg of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ;
- c) 6 mg of  $\text{H}_3\text{BO}_3$ ;
- d) 190 mg of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ;
- e) 3 mg of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ;
- f) 240 mg of  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ;
- g) 36 mg of  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ;
- h) 33 mg of  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ;
- i) 26 mg of  $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ .

Make up to 1 000 ml with water (6.1).

**6.2.2.3 Solution G (vitamin solution, optional).**

Dissolve in 100 ml of water (6.1) 0,6 mg of biotine, 2,0 mg of niacinamide, 2,0 mg of *p*-aminobenzoate, 1,0 mg of panthotenic acid, 10,0 mg of pyridoxal hydrochloride, 5,0 mg of cyanocobalamine, 2,0 mg of folic acid, 5,0 mg of riboflavin, 5,0 mg of DL-thioctic acid and 1,0 mg of thiamine dichloride or use a