
**Determination of the ultimate aerobic
biodegradability of plastic materials
in an aqueous medium — Method by
measuring the oxygen demand in a
closed respirometer**

*Évaluation de la biodégradabilité aérobie ultime des matériaux
plastiques en milieu aqueux — Méthode par détermination de la
demande en oxygène dans un respiromètre fermé*

(<https://standards.iteh.ai>)

Document Preview

[ISO 14851:2019](https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019)

<https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019>



iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[ISO 14851:2019](https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019)

<https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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.....	vi
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Principle.....	3
5 Test environment.....	3
6 Reagents.....	3
6.1 Distilled or deionized water.....	4
6.2 Test medium.....	4
6.2.1 Standard test medium.....	4
6.2.2 Optimized test medium.....	4
6.3 Pyrophosphate solution.....	6
6.4 Carbon dioxide absorber.....	6
7 Apparatus.....	6
8 Procedure.....	6
8.1 Test material.....	6
8.2 Reference material.....	7
8.3 Preparation of the inoculum.....	7
8.4 Test.....	8
9 Calculation and expression of results.....	9
9.1 Calculation.....	9
9.2 Expression and interpretation of results.....	10
10 Validity of results.....	10
11 Test report.....	11
Annex A (informative) Theoretical oxygen demand (ThOD).....	12
Annex B (informative) Correction of BOD values for interference by nitrification.....	13
Annex C (informative) Principle of a closed manometric respirometer.....	15
Annex D (informative) Two-phase closed-bottle version of the respirometric test.....	17
Annex E (informative) Example of the determination of a carbon balance.....	20
Annex F (informative) Example of a determination of the amount of water-insoluble polymer remaining at the end of a biodegradation test and the molecular mass of the polymer.....	22
Annex G (informative) Example of the determination of the CO₂ absorbed in the absorbent.....	23
Bibliography.....	25

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).

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.

The committee responsible for this document is ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*.

This second edition cancels and replaces the first edition (ISO 14851:1999), which has been technically revised. It also incorporates the Technical Corrigendum ISO 14851:1999/Cor.1:2005. The main changes compared to the previous edition are as follows:

- the footnotes have been renumbered;
- in [Annex C](#), errors in the key to [Figure C.1](#) have been corrected and minor improvements made to the figure itself;
- in scope and [Clause 8](#), soil and compost have been excluded for the inoculums used in this document;

- in 8.4, numbers of test flask for the test material and blank control have been changed from two to three;
- references in this document have been updated for latest active version;
- the Bibliography has been updated.

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.

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[ISO 14851:2019](#)

<https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019>

Introduction

With the increasing use of plastics, their recovery and disposal have become a major issue. As a first priority, recovery is promoted. Complete recovery of plastics, however, is difficult. For example, plastic litter, which comes mainly from consumers, is difficult to recover completely. Additional examples of plastics which are difficult to recover are fishing tackle, plastic microbeads in personal care products and water-soluble polymers. These plastic materials tend to leak from closed waste-management cycles into the environment. 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 are expected to be potentially biodegradable. Therefore, it is very important to determine the potential biodegradability of such materials and to obtain an indication of their biodegradability in natural environments.

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[ISO 14851:2019](https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019)

<https://standards.iteh.ai/catalog/standards/iso/c4cb1c7c-2c9a-4b8f-b10d-f685a1effe59/iso-14851-2019>

Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer

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 document specifies a method, by measuring the oxygen demand in a closed respirometer, for the determination of the degree of aerobic biodegradability of plastic materials, including those containing formulation additives. The test material is exposed in an aqueous medium under laboratory conditions to an inoculum from activated sludge.

If an unadapted activated sludge is used as the inoculum, the test simulates the biodegradation processes which occur in a natural aqueous environment; if a mixed or pre-exposed inoculum is used, the method is used to investigate the potential biodegradability of a test material.

The conditions used in this document do not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but this document is designed to determine the potential biodegradability of plastic materials or give an indication of their biodegradability in natural environments.

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

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[2]). 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 14851:2019(E)

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

activated sludge

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 in an activated sludge

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

biochemical oxygen demand

BOD

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

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

3.5

theoretical oxygen demand

ThOD

theoretical maximum 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.

3.6

total organic carbon

TOC

amount of carbon bound in an organic compound

3.7

dissolved organic carbon

DOC

part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 m·s⁻² 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, 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* (3.9)

3.9

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

3.10**biodegradation phase**

time, measured in days, from the end of the *lag phase* (3.8) of a test until about 90 % of the *maximum level of biodegradation* (3.9) has been reached

3.11**plateau phase**

time, measured in days, from the end of the *biodegradation phase* (3.10) until the end of a test

3.12**pre-exposure**

pre-incubation of an inoculum 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-conditioning**

pre-incubation of an inoculum 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

4 Principle

The biodegradability of a plastic material is determined using aerobic 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. The mixture is stirred in closed flasks in a respirometer for a period not exceeding 2 months. The carbon dioxide evolved is absorbed in a suitable absorber in the headspace of the flasks. The consumption of oxygen (BOD) is determined, for example by measuring the amount of oxygen required to maintain a constant volume of gas in the respirometer flasks, or by measuring the change in volume or pressure (or a combination of the two) either automatically or manually. An example of a respirometer is given in [Annex C](#). Alternatively, the two-phase closed-bottle version described in ISO 10708[3] may be used (see [Annex D](#)).

The level of biodegradation is determined by comparing the BOD with the theoretical amount (ThOD) and expressed in per cent. The influence of possible nitrification processes on the BOD has to be considered. The test result is the maximum level of biodegradation determined from the plateau phase of the biodegradation curve. Optionally, a carbon balance may be calculated to give additional information on the biodegradation (see [Annex E](#)). Moreover, also the absorbed carbon dioxide in the adsorber at the end of the test may be determined to give additional information on the biodegradation (see [Annex G](#)).

Unlike ISO 9408[6], which is used for a variety of organic compounds, this document is specially designed for the determination of the biodegradability 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 ± 1 °C, or at any other appropriate temperature depending on the inoculum used and the environment to be assessed.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or deionized water

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 may be used. For example, if simulating a natural environment use the standard test medium (6.2.1). If a test material is used at higher concentrations, use the optimized test medium (6.2.2) with higher buffering capacity and nutrient concentrations.

6.2.1 Standard test medium

6.2.1.1 Solution A

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

anhydrous potassium dihydrogen phosphate (KH_2PO_4)	8,5 g
anhydrous dipotassium hydrogen phosphate (K_2HPO_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 (NH_4Cl)	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;
- 1 ml of each of solutions B to D.

Make up to 1 000 ml with water (6.1). Prepare the test medium freshly before use. The solutions A up to C may be stored up to 6 months in the dark at room temperature.

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 test-material concentrations are used, increase the nitrogen content to keep the C:N ratio at about 40:1.

6.2.2.1 Solution A

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

anhydrous potassium dihydrogen phosphate (KH_2PO_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 (NH_4Cl)	2,0 g

6.2.2.2 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.

6.2.2.3 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.

6.2.2.4 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 (see second paragraph of 6.2.1.4).

6.2.2.5 Solution E (trace-element solution, optional)

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

70 mg of ZnCl_2 , 100 mg of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 6 mg of H_3BO_3 , 190 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 3 mg of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 240 mg of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 36 mg of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 33 mg of $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ and 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).

6.2.2.6 Solution F (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 solution of 15 mg of yeast extract in 100 ml of water (6.1). Filter the solution for sterilization using membrane filters (see 7.4).

Solutions E and F are optional and are not required if a sufficient concentration of the inoculum is used, for example, activated sludge. It is recommended that 1 ml portions be prepared and kept refrigerated until use.

6.2.2.7 Preparation

To prepare 1 l of test medium, add, to about 800 ml of water (6.1):

- 100 ml of solution A;
- 1 ml of each of solutions B to D and, optionally, E and F.

Make up to 1 000 ml with water (6.1) and measure the pH.