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Introduction

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Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	



DRAFT International Standard

ISO/DIS 18957

Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions in laboratory

*Plastiques — Détermination de la biodégradation aérobie des
matières plastiques exposées à l'eau de mer en utilisant des
conditions accélérées en laboratoire*

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ISO/TC 61/SC 14

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2025-01-06

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2025-03-31

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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).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

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

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

Plastics have been used in various fields because of their highly durable characteristics. Plastics are also used intentionally in the environment or in applications where unintentional loss to the environment is unavoidable. The degree of biodegradation of plastics in natural environment is of interest in order to better characterize the behaviour of plastics in these very particular environments. Thus, the test methods to measure the degree and rate of biodegradation are of major interest to obtain an indication of the potential biodegradability of plastic materials when exposed to different marine habitats.

The biodegradability of plastics is defined in laboratory tests, for example ISO 18830, ISO 19679, ISO 23977-1 and ISO 23977-2, but these test methods in a laboratory are basically using only seawater and sediment.

Since existing test methods are not intended to shorten the test period, the test period often extends to one year and it is sometimes difficult to assess the degree of biodegradability of the sample in a short period of time.

This document provides an accelerated test method for determining the biodegradation level of plastics exposed to seawater in laboratory in shorter time by increasing the number of microorganisms in seawater and enriching the flora, by adding nutrients, pre-treatment by sediment, and condensation of seawater. The degree of biodegradation is calculated based on measurement of the oxygen (O₂) demand in a closed respirometer or carbon dioxide (CO₂) evolution.

Test material is taken up by microorganisms, metabolized, and finally mineralized to CO₂. Part of the carbon originating from the test material is converted to biomass. The biomass cannot be detected unless the bacteria die and undergo self-digestion, and it takes longer period of time than the conversion to biomass and it may require long test period to complete the mineralization of the test material. On the other hand, since biomass components are produced by the microbial activity, the total biodegradation considered by carbon flow can be estimated by the sum of the degree of biodegradation based on O₂ and/or CO₂ analysis and the degree of conversion to biomass. Therefore, for the purpose of promoting the development of biodegradable plastics, this document also provides a method for measuring the biomass components produced by metabolizing the test resin before it is completely mineralized to CO₂, that enables to evaluate the potential biodegradability considered by carbon flow in much shorter period of time than full mineralization.

Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions in laboratory

1 Scope

This document describes a laboratory test method to determine the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions.

Furthermore, this document describes the general requirements of the apparatus and the procedures for using this test method. The biodegradation is determined by measuring the oxygen demand in a closed respirometer or by measuring the CO₂ evolved during mineralization of the plastic materials by microorganisms in the seawater.

This test method is designed to give an early indication of the potential biodegradability of plastic materials in marine environment.

In addition, for the purpose of promoting the development of biodegradable plastics, this document also provides a method for measuring the biomass components produced by metabolizing the test resin before it is completely mineralized to CO₂, that enables to evaluate the potential biodegradability considered by carbon flow by the sum of the degree of biodegradation based on O₂ and/or CO₂ analysis and the degree of conversion to biomass in much shorter period of time than full mineralization.

This method is not suitable to assess the degree of disintegration of plastic materials caused by abiotic factors like heat or UV radiation.

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 472, *Plastics — Vocabulary*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

ISO 10210, *Plastics — Methods for the preparation of samples for biodegradation testing of plastic materials*

ISO 10523, *Water quality — Determination of pH*

ISO 11261, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method*

ISO 14851, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer*

ISO 18830, *Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface — Method by measuring the oxygen demand in closed respirometer*

ISO 19679, *Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface — Method by analysis of evolved carbon dioxide*

ISO 22404, *Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide*

ISO 23977-1, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide*

ISO 23977-2, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology 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

pelagic zone

water body above the seafloor

Note 1 to entry: It is also referred to as the open water or the water column.

Note 2 to entry: The surface of the pelagic zone is moved by wind-driven waves, is in contact with the atmosphere and exposed to sunlight. With increasing depth pressure increases, temperature decreases, and light and surface wave energy are attenuated.

[SOURCE: ISO 22766:2020, 3.4]

3.2

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.

[SOURCE: ISO 18830:2016, 3.1]

3.3

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.

[SOURCE: ISO 18830:2016, 3.2]

3.4

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 17556:2019, 3.14]

3.5 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⁻² for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

[SOURCE: ISO 14852:2021, 3.7]

3.6 proton NMR ¹H NMR

nuclear magnetic resonance using proton (¹H NMR) as the observed nucleus

3.7 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* (3.8)

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.8]

3.8 biodegradation phase

time from the end of the *lag phase* (3.7) of a test until the plateau phase has been reached

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.10]

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.

[SOURCE: ISO 14852:2021, 3.9]

3.10 plateau phase

time from the end of the *biodegradation phase* (3.8) until the end of a test

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.11]

4 Principle

This document describes a test method for determination of the aerobic biodegradation of plastic materials exposed in seawater using accelerated conditions in laboratory. The biodegradation is determined by measuring the oxygen demand in a closed respirometer or by measuring the CO₂ evolved during mineralization of the plastic materials by microorganisms in the seawater.

The biochemical oxygen demand (BOD) is determined by consumption of oxygen, 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.

The level of biodegradation is calculated by dividing the BOD by the theoretical oxygen demand (ThOD) and is expressed in percentage. The influence of possible nitrification processes on the BOD shall be considered. The test result is the maximum level of biodegradation determined from the plateau phase of the biodegradation curve.

To accelerate the biodegradation test using seawater, different methods are applied, such as adding nutrients to the seawater, changing the incubation temperature, washing the seawater with marine sediment, and concentrating the microorganisms via a filtration-based protocol.

In general, the smaller the test material/seawater ratio, the higher the biodegradation rate. To prevent a limitation of the aerobic biodegradation rate of the test material due to low metabolic rates of microorganisms, it is important to guarantee sufficient oxygen transfer into the seawater phase. The larger the surface area of the sample, the faster the biodegradation. In addition, at higher test temperatures chemical reactions and biological processes are faster, often resulting in higher biodegradation rates. However, the test temperature should not exceed 30 °C since the bacterial strain composition in seawater might be negatively affected with regard to biodegradation capability.

Increase the capability of seawater microorganisms for plastic biodegradation is mainly achieved by increasing the number of microorganisms in seawater and increasing diversity. However, even if the microorganisms are enriched, their capability to degrade plastic materials might be limited, e.g., by nutrient limitations in the seawater, which should be addressed by maintaining a minimum amount of inorganic nutrients.

Since oxygen consumption and CO₂ generation basically correspond to each other, the amount of CO₂ evolved may be measured instead of the BOD test.

Test material is taken up by microorganisms, metabolized, and finally mineralized to CO₂. Part of the carbon originating from the test material is converted to biomass. The biomass cannot be detected unless the bacteria die and undergo self-digestion, and it takes longer period of time than the conversion to biomass and it may require long test period to complete the mineralization of the test material. On the other hand, since biomass components are produced by the microbial activity, the total biodegradation considered by carbon flow can be estimated by the sum of the degree of biodegradation based on O₂ and/or CO₂ analysis and the degree of conversion to biomass.

For material development it is highly important to understand if a material is fully biodegradable. Therefore, it is important to understand where the carbon goes if it cannot be detected by sole CO₂ evolution measurement. This document also provides a method for measuring the biomass components produced by metabolizing the test resin before it is completely mineralized to CO₂, that enables to evaluate the potential biodegradability considered by carbon flow in much shorter period of time than full mineralization.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an environment which is free from vapor inhibitory to marine microorganisms, and which is maintained at a constant mesophilic temperature. The temperature shall be between 15 °C to 30 °C, preferably at 27 °C, but not exceeding 30 °C, to an accuracy of ±1 °C and shall be monitored through a data logger and clearly indicated in the test report.

NOTE Test results are obtained for temperatures that can be different from real conditions in marine environment.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Water

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