



**International
Standard**

ISO 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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Sample Document

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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 document 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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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 basically use 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 the contained carbon finally mineralized to CO₂. Part of the carbon originating from the test material is temporarily converted to biomass. The biomass cannot be detected unless the bacteria die and undergo self-digestion. It takes a longer period of time than the conversion to biomass and it may require a 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 material 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. It describes the general requirements of the apparatus and the procedures for using this test method.

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

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

ASTM D1141-98, *Standard Practice for Preparation of Substitute Ocean Water*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472 and the following 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

carbon flow

series of compounds produced from carbon derived from plastics during biodegradation

Note 1 to entry: The series of compounds includes polymers with reduced molecular weight, water-soluble oligomers, compounds in the process of biochemical metabolism, organic matter that makes up bacterial cells, and carbon dioxide that is produced when plastics are completely biodegraded.

3.2

¹H NMR

proton NMR

nuclear magnetic resonance using proton as the observed nucleus

3.3

pelagic seawater

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]

3.4

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* (3.10)

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.8]

3.5

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]

3.6

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]

3.7

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]

3.8
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 $40\,000\text{ m}\cdot\text{s}^{-2}$ for 15 min or by membrane filtration using membranes with pores of $0,2\text{ }\mu\text{m}$ to $0,45\text{ }\mu\text{m}$ diameter.

[SOURCE: ISO 14852:2021, 3.7]

3.9
theoretical amount of evolved carbon dioxide
ThCO₂

maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula

[SOURCE: ISO 23977-1:2020, 3.3, modified – Note 1 to entry was removed.]

3.10
biodegradation phase

time from the end of the *lag phase* ([3.4](#)) 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]

3.11
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]

3.12
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.

[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 31 °C since the bacterial strain composition in seawater might be negatively affected with regard to biodegradation capability. See [Annex B](#) for examples of biodegradation BOD test with different temperature.

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 the contained carbon finally mineralized to CO₂. Part of the carbon originating from the test material is temporarily 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 material 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, and which is maintained at a constant mesophilic temperature. The temperature shall be between 15 °C to 31 °C, preferably at 27 °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, free of toxic substances and containing less than 2 mg/l of TOC.

6.2 Natural seawater/sediment

The seawater sample shall be used for testing preferably on the day the water is collected, or it can be stored in dark between 4 °C to 13 °C and be used for testing within 3 days. If the seawater is kept at 4 °C, it can be used for testing for 2 weeks. Sediment can also be stored if it is kept at 4 °C and regularly mixed and aerated to avoid anaerobic conditions.

Prior to use, remove coarse particles from the seawater and, if applicable, from the sediment by appropriate means, e.g., by sieving with a mesh of 3 mm opening. The procedure used shall be reported.

Seawater can be filtered using a laboratory filter paper having particle retention between 1 µm to 5 µm to remove particles.

Measure TOC, pH and nitrogen content of seawater and, if applicable, of sediment samples in accordance with ISO 8245, ISO 10523 and ISO 11261, respectively, and measure salinity (PSU) by means of, for example, refractometer-type and electrical conductivity-type salinity meter.

If the TOC content of the seawater sample is found to exceed about 20 % of the theoretical TOC after the addition of the test item, the seawater should not be used, or be diluted with artificial seawater.

If artificial seawater is used, it shall be prepared in accordance with ASTM D1141-98.

Provide the following information on the seawater, and, if applicable, on the sediment sample itself:

- date of collection;
- depth of collection (m);
- appearance of sample – turbid, clear, etc.;
- temperature at the time of collection (°C);
- salinity (PSU);
- total organic carbon (TOC; mg/l);
- nitrogen (total-N; mg/l);
- pH;
- description of the pre-conditioning process, including location and storage, if applicable.¹⁾

7 Apparatus

Ensure that all glassware shall be thoroughly cleaned and dry, in particular, free from organic or toxic matter. Required is usual laboratory equipment, plus the following.

7.1 Detection system for calculation of biodegradability

7.1.1 General

In the case of using BOD measurement for calculation of biodegradability, a closed respirometer (7.1.2) is used. In the case of using CO₂ production measurement for calculation of biodegradability, a CO₂ production measurement system (7.1.3) is used.

7.1.2 Closed respirometer for static BOD measurement

Flasks of the volume of about 300 ml to 500 ml are appropriate. The vessels shall be located in a constant temperature room or in an apparatus fitted with a thermostat (e.g. water-bath).

Flasks with higher or lower volumes can be used, if it satisfies the volume condition specified in (see 8.1) and the experimental conditions in the flask are not affected.

A suitable apparatus is shown in [Annex A, Figure A.1](#).

Any closed respirometer able to determine with sufficient accuracy the biochemical oxygen demand is suitable, preferably an apparatus which measures and replaces automatically and continuously the oxygen consumed so that no oxygen deficiency and no inhibition of the microbial activity occurs during the degradation process. Analytical equipment to measure total organic carbon (TOC) and dissolved organic carbon (DOC) is given in ISO 8245.

7.1.3 Analytical equipment for determining producing carbon dioxide

Any test flask able to determine with sufficient accuracy the biochemical evolution of carbon dioxide is suitable in accordance with ISO 14852, or ISO 23977-1, or ISO 19679. The evolved CO₂ can be quantitatively measured also using other suitable methods such as those based on infrared CO₂-analysers or those based on TOC analysers equipped with an infrared photometer or on gravimetric analysis.

7.1.4 Analytical balance, which shall have a sensitivity of at least 0,1 mg.

7.1.5 Magnetic stirrer, which shape, and size are described in Annex A as example.

7.1.6 pH meter.

7.2 Analytical equipment for measuring nitrate and nitrite concentrations (optional)

In case the test material contains nitrogen, a quantitative test to determine nitrate/nitrite in the medium before and after the test is recommended by means of ion chromatography in accordance with ISO 10304-1.

7.3 Analytical equipment for measuring remaining test material and degradation products (optional)

To calculate potential of biodegradation considered by carbon flow (9.4), a quantitative test to determine remaining test material and degradation products in the medium after the test is recommended by means of ¹H NMR which carrier frequency for proton (¹H) should be 300 MHz or more in accordance with JIS K 0138:2018.

8 Procedure**8.1 Test material**

The test material shall be of known mass and contain sufficient carbon to yield a BOD that can be adequately measured by the chosen system [closed respirometer (7.1)].

Use a test material concentration of at least 75 mg/l up to 300 mg/l of seawater. The mass of test material should correspond to a ThOD of about 126 mg/l or a TOC of about 45 mg/l. The headspace of the flask should be 100 ml and more per 10 mg sample to ensure that air is adequately supplied to the sample.

The ThOD (see ISO 14851:2019, Annex A) and the TOC (using ISO 8245, ISO 10694 or from the chemical formula or determine by elemental analysis) shall be calculated.

Add KH₂PO₄ (11,0 mg/l) and NH₄Cl (9,55 mg/l) to seawater at the beginning of a test.

If necessary, KH₂PO₄ can be added up to 100 mg/l and NH₄Cl can be added up to 50 mg/l. However, when ammonium chloride is added at a high concentration, the oxygen consumption due to nitrification may differ between the blank and the test system, so after the test, NO₂ and NO₃ ions shall be analysed then corrected as mentioned in 9.2 for BOD measurement.

An experimental example of the production of NO_x ions after biodegradation in the presence of ammonium chloride is shown in Annex D.

Following ratio of the test material is recommended: