
**Plastics — Determination of the
aerobic biodegradation of plastic
materials exposed to seawater —**

**Part 2:
Method by measuring the oxygen
demand in closed respirometer**

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*Plastiques — Détermination de la biodégradation aérobie des
matières plastiques exposées à l'eau de mer —*

*Partie 2: Méthode par mesure de la demande en oxygène dans un
respiromètre fermé*

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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).

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 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*.

A list of all parts in the ISO 23997 series can be found on the ISO website.

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

According to the United Nations Environment Program (UNEP), one of the most notable properties of synthetic polymers and plastics is their durability which, combined with their accidental loss, deliberate release and poor waste management has resulted in the ubiquitous presence of plastic in oceans (UNEP, 2015^[15]).

It is well known and documented that marine litter can pose risks and a negative impact on living marine organisms and on human beings. Degradability of plastic materials exposed to the marine environment is one of the factors affecting impact and strength of effects. The uncontrolled dispersion of biodegradable plastics in natural environments is not desirable. The biodegradability of products cannot be considered as an excuse to spread wastes that should be recovered and recycled. However, test methods to measure rate and level of biodegradation in natural environments are of interest in order to better characterize the behaviour of plastics in these very particular environments. Thus, the degree and rate of biodegradation is of major interest in order to obtain an indication of the potential biodegradability of plastic materials when exposed to different marine habitats.

ISO/TC 61/SC 14 has established several test methods for biodegradation testing of plastic materials under laboratory conditions covering different environmental compartments and test conditions, as shown in [Table 1](#).

Table 1 — Test methods for biodegradation testing of plastics

Environmental compartment	Conditions Presence/absence of oxygen	Test methods
Controlled composting conditions	Aerobic conditions	ISO 14855-1
		ISO 14855-2
High-solids anaerobic-digestion conditions	Anaerobic conditions	ISO 15985
Controlled anaerobic slurry system	Anaerobic conditions	ISO 13975
Soil	Aerobic conditions	ISO 17556
Aqueous medium	Aerobic conditions	ISO 14851
	Anaerobic conditions	ISO 14852
Seawater/sandy sediment interface	Aerobic conditions	ISO 18830 ^a
		ISO 19679 ^a
Marine sediment	Aerobic conditions	ISO 22404 ^a
Seawater	Aerobic conditions	ISO 23977-1 ^a
		ISO 23977-2 ^a

^a Test method for measuring biodegradation of plastic materials when exposed to marine microbes.

All marine biodegradation test methods are based on exposure of plastic materials to marine samples (seawater and/or sediment) taken from shoreline areas. By a quantitative viewpoint, these methods are not equivalent, because, for example, the microbial density in seawater is generally lower compared to the density determined in sediment. In addition, the microbial composition and diversity can be different. Moreover, as a rule, the nutrient concentration found in sediment is normally higher compared to the concentration in seawater.

This document provides a test method for determining the biodegradation level of plastic materials exposed to the microbial population present in seawater from a pelagic zone under laboratory conditions. The biodegradation is followed by measuring the oxygen demand in a closed respirometer.

The test is performed with either seawater only (“pelagic seawater test”) or with seawater to which little sediment was added (“suspended sediment seawater test”).

The pelagic seawater test simulates the conditions found in offshore areas with low water currents and low tidal movements, whereas the suspended sediment seawater test simulates conditions which might be found in coastal areas with stronger water currents and tidal movements.

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Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater —

Part 2: Method by measuring the oxygen demand in closed respirometer

1 Scope

This document specifies a laboratory test method for determining the degree and rate of the aerobic biodegradation level of plastic materials. Biodegradation of plastic materials is determined by measuring the oxygen demand in a closed respirometer when exposed to seawater sampled from coastal areas under laboratory conditions.

The conditions described in this document might not always correspond to the optimum conditions for the maximum degree of biodegradation, however this test method is designed to give an indication of the potential biodegradability of plastic materials.

NOTE This document addresses plastic materials but can also be used for other materials.

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 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*

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 <http://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]

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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:—, 3.7]

3.6 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:—, 3.8]

3.7 biodegradation phase

time from the end of the *lag phase* (3.6) of a test until the plateau phase has been reached

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:—, 3.10]

3.8**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:—, 3.9]

3.9**plateau phase**

time from the end of the *biodegradation phase* (3.7) until the end of a test

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:—, 3.11]

3.10**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

[SOURCE: ISO 14852:—, 3.13]

4 Principle**iTeh STANDARD PREVIEW**

This document describes two variations of a test method for determining the biodegradability of plastic materials by the indigenous population of microorganisms in natural seawater using a static aqueous test system. The test is performed under mesophilic test conditions for up to two years by incubating plastic materials with either seawater only (“pelagic seawater test”) or with seawater to which low amount of sediment has been added (“suspended sediment seawater test”), coming from the same site as that from which the seawater was taken.

The system is contained in a closed flask, in a respirometer. 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.

The level of biodegradation is determined by comparing the BOD with the theoretical amount (ThOD) and 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.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an enclosure which is free from vapours inhibitory to marine microorganisms and which is maintained at a constant mesophilic temperature. It should preferably be between 15 °C to 25 °C, but not exceeding 28 °C, to an accuracy of ±1 °C. Any change in temperature shall be justified 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.

6.2 Natural seawater/sediment

Sampling, preservation, handling, transport and storage of natural seawater, and, if applicable, sediment collected from the same site as that from which the seawater is taken, shall be in accordance with ISO 5667-3.

Prior to use, remove coarse particles from the seawater and, if applicable, from the sediment by appropriate means. The procedure used shall be reported.

Seawater can be filtered using a paper filter in order to remove coarse particles. It is recommended to reduce the amount of coarse particles in sediment by means of at least two washing steps using filtered seawater without coarse particles.

Measure TOC, pH and nitrogen content of seawater and, if applicable, of sediment samples according to ISO 8245, ISO 10523 and ISO 11261, respectively.

If the TOC content of the seawater sample is found to be high, the seawater should be pre-conditioned for about a week prior to use. If, for instance, the background concentration of TOC exceeds about 20 % of the total TOC after addition of the test item, then pre-condition the seawater and, if applicable, the sediment by stirring under aerobic conditions at the test temperature and in the dark or in diffuse light in order to reduce the content of easily degradable organic material.

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, if applicable.

7 Apparatus

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

7.1 Closed respirometer.

Flasks of the volume of about 300 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).

Reactors with higher or lower volumes can be used, if environmental conditions are not affected.

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

Any 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.

7.2 Analytical equipment for measuring nitrate and nitrite concentrations.

A qualitative test (for example test strips or photometric tests for nitrate and nitrite) is recommended first to decide if any nitrification has occurred. If there is evidence of nitrate/nitrite in the medium, a quantitative determination using a suitable method (for example ion chromatography) is required.

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

7.4 **Magnetic stirrer**.

7.5 **pH meter**.

8 Procedure

8.1 Test material

The sample 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 100 mg/l of seawater plus sediment. The mass of the samples should correspond to a ThOD of about 170 mg/l or a TOC of about 60 mg/l. The maximum mass of sample per flask is limited by the oxygen supply to the respirometer.

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

The test material is added to a test flask, either as powder or in the form of a film. If the test material is used in the form of powder, particles of known, narrow size distribution should be used. A particle-size distribution with a maximum diameter of 250 µm is recommended. The preparation of powder shall be performed in accordance with ISO 10210. If the test material is used in the form of a film, it can be added either as pieces in the range of 0,2 cm × 0,2 cm to 0,5 cm × 0,5 cm or as a single plastic strip (width: approximately 1,0 cm, length: depending on weight of the polymer and thickness of the film). It is recommended that the plastic strip is fixed in, for example, a polytetrafluoroethylene (PTFE) coated fibre net¹⁾ (size: approximately 4 cm × 9 cm, mesh size: 5 mm × 5 mm). The fibre net is folded into 2 layers (approximately 2 cm × 9 cm) with the plastic strip test material fixed in between. Then, the two ends of the fibre net are attached together. The test material fixed between the fibre net is placed upright on the ground of a bottle base in the form of a cylinder (see Annex A, Figure A.2).

The form and shape of the test material can influence its biodegradability. Similar particle sizes of power should preferably be used in the test. Similar shapes and thicknesses of the films should preferably be used if different kinds of plastic materials are to be compared.

When powder or pieces of films are used in the test, particles or film pieces can stick on the inner wall of the testing bottle above the seawater. In such cases, a slight manual shaking of the bottle is recommended to regain the powder or film pieces back to the seawater sample. If the material is added as a cylindrical plastic strip fixed between, for example, a polytetrafluoroethylene (PTFE) coated fibre net (see Annex A, Figure A.2), it is immersed in the seawater most of the time.

1) PTFE Glass Fabric (product no 9002) produced by Fiberflon (<https://www.fiberflon.de/Products/PTFE-Coated-Open-Mesh-Fabrics/Page-307-17.aspx>) has been found satisfactory for this purpose and is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.