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

*Plastiques — Détermination de la biodégradation aérobie des
matières plastiques non-flottantes dans une interface eau de mer/
sédiments — 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).

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.

The committee responsible for this document is 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 second edition cancels and replaces the first edition (ISO 19679:2016), which has been technically revised.

The main changes compared to the previous edition are as follows:

- in [Annex A](#): Density of O₂ in air at 1 atm, 28 °C and a relative humidity of 100 % has been corrected and the subsequent calculations have been adapted accordingly.

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

Products made with biodegradable plastics are designed to be recovered by means of organic recycling in composting plants or in anaerobic digesters. 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 (such as soil or the marine environment) are of interest in order to better characterize the behaviour of plastics in these very particular environments. As a matter of fact, some plastics are used in products that are applied in the sea (e.g. fishing gear) and sometimes they can get lost or put willingly in the marine environment. The characterization of biodegradable plastic materials can be enlarged by applying specific test methods that enable the quantitative assessment of biodegradation of plastics exposed to marine sediment and seawater. Plastic products are directly littered or arrive with fresh waters in the pelagic zone (free water). From there, and depending on density, tides, currents, and marine fouling plastics can sink to the sublittoral, and reach the seafloor surface. Many biodegradable plastics have a density higher than 1 and therefore tend to sink. The sediment passes from aerobic to anoxic and finally anaerobic conditions going from the surface (the interface with seawater) into deeper layers, displaying a very steep oxygen gradient.

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

1 Scope

This document specifies a test method to determine the degree and rate of aerobic biodegradation of plastic materials when settled on marine sandy sediment at the interface between seawater and the seafloor, by measuring the evolved carbon dioxide (CO₂). This test method can also be applied to other solid materials.

This test method is a simulation under laboratory conditions of the habitat found in different seawater/sediment-areas in the sea, e.g. in a benthic zone where sunlight reaches the ocean floor (photic zone) that, in marine science, is called sublittoral zone

The determination of biodegradation of plastic materials and other solid materials buried in marine sediment is outside the scope of this document.

NOTE Measurement of aerobic biodegradation can also be obtained by monitoring the oxygen consumption, as described in ISO 18830.

The conditions described in this document do not always correspond to the optimum conditions for the maximum degree of biodegradation to occur.

2 Normative references

There are no normative references in this document.

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

theoretical amount of evolved carbon dioxide

ThCO₂

maximum theoretical amount of carbon dioxide evolved after completely oxidising a chemical compound, calculated from the molecular formula or from determination of *total organic carbon (TOC)* (3.2)

Note 1 to entry: It is expressed as mg of carbon dioxide evolved per mg or g of test compound.

3.2

total organic carbon

TOC

amount of carbon bound in an organic compound

Note 1 to entry: Total organic carbon is expressed as mg of carbon per 100 mg of the compound.

3.3 dissolved organic carbon DOC

part of the organic carbon in water which cannot be removed by specified phase separation methods, for example by centrifugation at $40\,000\text{ ms}^{-2}$ for 15 min or by membranes with pores of $0,2\text{ }\mu\text{m}$ to $0,45\text{ }\mu\text{m}$ diameter

3.4 pre-conditioning phase

pre-incubation of an inoculum under the conditions of the subsequent test in the absence of test material, with the aim to consume potential organic matter present in excess that could disturb biodegradation measurement and to improve the acclimatization of the microorganisms to the test conditions

4 Principle

This test method is based on the determination of evolved CO_2 and derives from ISO 14852. The testing medium is based on a solid phase and a liquid phase. The solid phase is a sandy marine sediment laid in the bottom of a closed flask; the liquid phase is a column of natural or artificial sea water, poured on the sediment. The test material is preferably in the form of a film to be laid down on top of the sediment, at the interface between the solid phase and the liquid phase. This is a simulation of an object that has sunk and finally reached the sea floor. The system is contained in a closed flask.

The CO_2 evolved during the microbial degradation is determined by a suitable analytical method. The level of biodegradation is determined by comparing the amount of CO_2 evolved with the theoretical amount (ThCO_2) and expressed in percentage. The test result is the maximum level of biodegradation, determined from the plateau phase of the biodegradation curve. The principle of a system for measuring evolved CO_2 is given in ISO 14852:2018, Annex A.

The details of interlaboratory testing based on the test method specified in this document are available in Reference [6].

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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 $15\text{ }^\circ\text{C}$ to $25\text{ }^\circ\text{C}$, but not exceeding $28\text{ }^\circ\text{C}$, to an accuracy of $\pm 2\text{ }^\circ\text{C}$. Any change in temperature shall be justified and clearly indicated in the test report.

NOTE Temperatures applied in the test can be different from those found in marine environments

6 Reagents

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

6.2 Artificial seawater.

Dissolve:

Sodium chloride (NaCl)	22 g
Magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)	9,7 g
Sodium sulfate (Na_2SO_4)	3,7 g

Calcium chloride (CaCl ₂)	1 g
Potassium chloride (KCl)	0,65 g
Sodium hydrogen carbonate (NaHCO ₃)	0,20 g

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

6.3 Natural seawater/sediment.

Take a sample of a sandy sediment and seawater with a shovel beneath the low-water line into a bucket. Transfer the wet sediment together with seawater into sealed containers for transport and fast deliver it to the laboratory. After delivery, conserve the sediment at low temperature (approximately 4 °C) until use. The seawater/sediment sample should be preferably used within 4 weeks after sampling. Record storage time and conditions.

NOTE Seawater and sediment can also be sampled from large, well-running public marine aquaria.

Measure the TOC, pH and nitrogen content of the sediment and of the natural seawater if used instead of artificial seawater. The carbon content of sediment should be in the range of 0,1 % to 2 %.

A preliminary oxidation can be applied to the sediment in order to decrease the organic matter content and the background respiration. Sediment and seawater are fluxed with air and gently stirred (max. 20 r/min to 30 r/min) in a large container for the desired period of time. Include this pre-treatment process in the test report.

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7 Apparatus

7.1 Test flasks.

Biometer flasks of the volume of about 250 ml are appropriate. Reactors with higher volumes can be used, if test conditions are not affected. The vessels shall be located in a constant-temperature room or in a thermostatic apparatus (e.g. water-bath). Stirring can be applied on seawater on condition that it does not disturb the sediment/seawater interface.

NOTE A suitable apparatus is shown in [Figure A.1](#). An example of a stirred apparatus is given in OECD TG 308: 2002, Annex 4^[2].

7.2 Container for the CO₂ absorber.

A glass beaker to be located in the headspace of the reactor and filled with 10 ml of Ba(OH)₂ 0,0125 mol/l or with 3 ml of KOH 0,5 mol/l.

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

7.4 **pH meter**.

8 Procedure

8.1 Test material

The test material should be in film or sheet form. Cut samples of the test material in the shape of a disk. Disks shall have a smaller diameter than the glass flasks, so that the disks can be easily laid on the bottom of the glass flask.

The sample shall be of known mass and contain sufficient carbon to yield CO₂ that can be adequately measured by the system used.

Use a test material concentration of at least 100 mg/l of seawater plus sediment. This mass of the sample should correspond to a TOC of about 60 mg/l. The maximum mass of sample per flask is limited by the oxygen supply to the glass flask. A test material concentration of 150 mg/l to 300 mg/l of seawater plus sediment is recommended.

Calculate the TOC from the chemical formula or determine it by a suitable analytical technique (e.g. elemental analysis or measurement in accordance with ISO 8245) and calculate the ThCO_2 .

The form and shape of the test material may influence its biodegradation. Similar shapes and thicknesses should preferably be used if different kinds of plastic materials are to be compared.

NOTE When the test material in form of film is laid down on the surface of the sediment, it can limit the gas exchange between the water body and the sediment, promoting the formation of anaerobic zones under the test material. In order to reduce this effect, it is possible to perforate the film sample homogeneously over the entire surface.

8.2 Reference material

Use ashless cellulose filters as a reference material¹⁾. If possible, the TOC, form, and size should be comparable to that of the test material. As a negative control, a non-biodegradable polymer (such as polyethylene) in the same form as the test material shall be used.

8.3 Preparation of the sediment

Filter the sediment in a funnel with a coarse filter paper to eliminate excess seawater. Sediment is ready for testing when dripping of sea water is ended. Sediment after filtering is named "wet sediment" hereafter.

8.4 Test setup

Provide several flasks, so that the test includes at least the following:

- a) three flasks for the test material (symbol F_T);
- b) three flasks for the blank (symbol F_B);
- c) three flasks for reference material (symbol F_C);
- d) three flasks for negative control (symbol F_N).

Two flasks for test material, blank, reference material, and negative control may be used instead of three for screening purposes.

8.5 Pre-conditioning phase

In a typical case, use a test flask with a volume of 250 ml. Put 30 g of the wet sediment on the bottom of the flask. Carefully pour 70 ml of natural or artificial seawater. Reactors with higher volumes can be used, if test conditions are not affected. The test should be performed with a water/sediment volume ratio between 3:1 and 5:1 and a sediment layer of about 0,3 cm to 0,5 cm, depending on the granulometry of the sediment.

When using very coarse-grained sediment, the layer can be increased up to 1,5 cm.

Add CO_2 absorber to the absorber compartments of the test flask in a typical case 3 ml of KOH 0,5 mol/l or 10 ml of $\text{Ba}(\text{OH})_2$ 0,0125 mol/l. Place the flasks in a constant-temperature environment and allow all vessels to reach the desired temperature. Take the necessary readings and monitor the CO_2 evolution.

1) Laboratory filter paper Whatman n° 42 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.

This phase is carried out in order to verify that the endogenous respiration is similar in the different vessels and also to obtain a preliminary oxidation of excess organic matter, in order to start the test with a lower endogenous respiration. The inoculum can be gently stirred in order to accelerate the biodegradation of excess organic matter.

This phase is generally protracted for a week but is possible to extend this time if a high amount of CO₂ evolved is measured.

In case the CO₂ evolution of a vessel is different, reject the diverging vessel or in case of multiple anomalies, start again using new sediment.

8.6 Start of the test

Dunk the plastic film sample, cut as described in [8.1](#), on the sediment of each vessel. Mass of samples (test and reference material) should be about 20 mg each when using a flask with a volume of 250 ml corresponding to an initial test item concentration specified in [8.1](#). In order to ensure a homogeneous contact between sample and sediment, it is recommended to cover the sample with a suitable cover slip. The cover slips shall also be introduced in blank vessels, for assuring similar conditions.

NOTE A suitable cover slip can be made using a common non-biodegradable vinyl-coated fibreglass mosquito net with a fibre diameter of about 280 µm and a 1,8 mm × 1,6 mm mesh.

For an example, see [Annex A](#).

Repeat the procedure for the reference material and the material for the negative control to the respective flasks. Record the mass of the sediment, the sample and the volume of seawater introduced in each vessel.

Nutrients may be supplemented as needed to support microbial diversity and to maintain the capacity to biodegrade the test material. The need and timing of additional nutrients or other appropriate measures may be judged by observation of the temporal course of the biodegradation of the reference substance cellulose. Any addition and the applied method shall be reported in the test report.

8.7 Carbon dioxide measurement

8.7.1 The CO₂ reacts with Ba(OH)₂ and precipitates as BaCO₃. The amount of CO₂ produced is determined by titrating the remaining Ba(OH)₂ with 0,05 mol/l HCl to a phenolphthalein end-point or by automatic titrator. Because of the static incubation, the BaCO₃ builds up on the surface of the liquid and shall be broken up periodically by shaking the container gently to ensure continued absorption of the evolved CO₂. This problem can be avoided by using KOH instead of Ba(OH)₂, which does not form a precipitate.

NOTE A discussion on the use of KOH in place of Ba(OH)₂ is reported in Reference [\[5\]](#).

8.7.2 The containers for the CO₂ absorber shall be removed and titrated before their capacity is exceeded. The period of time will vary with sediments and test materials and increases slowly as the carbon content of the sediment is reduced (a recommended frequency of every 3 to 4 days for the first 2 to 3 weeks and every 1 to 3 weeks, thereafter). At the time of removal of the containers, the reactor should be allowed to sit open so that the air is refreshed before replacing 10 ml of fresh Ba(OH)₂ and resealing the reactor. The reactors should remain open approximately 15 min.

8.7.3 The CO₂ evolution rate may reach a plateau when all of the accessible carbon has been oxidized. The test may be terminated at this point or earlier, at the discretion of the user. If possible, the residual test material may be extracted from the sediment with an appropriate method and quantified (optional).

NOTE 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.