
**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 à l'interface eau de mer/sédiments
— Méthode par analyse du dioxyde de carbone libéré*

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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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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For an explanation on 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 5, *Physical-chemical properties*.

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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 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 may 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 International Standard 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.

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 buried in marine sediment is outside the scope of this International Standard.

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

The conditions described in this International Standard may not always correspond to the optimum conditions for the maximum degree of biodegradation to occur.

2 Normative references

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The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14852:1999, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide*

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.

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)

Note 1 to entry: It is expressed as milligrams of carbon dioxide evolved per milligram or gram 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 milligrams of carbon per 100 mg of the compound.

3.3 dissolved organic carbon DOC

that part of the organic carbon in water which cannot be removed by specified phase separation methods, for example by centrifugation at 40 000 ms⁻² for 15 min or by membranes with pores of 0,2 µm to 0,45 µm 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 carbon dioxide 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 carbon dioxide evolved during the microbial degradation is determined by a suitable analytical method. The level of biodegradation is determined by comparing the amount of carbon dioxide evolved with the theoretical amount (ThCO₂) 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 carbon dioxide is given in ISO 14852:1999, Annex A.

The details of interlaboratory testing based on the test method specified in this International Standard are available in Reference [5].

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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 °C to 25 °C, but not exceeding 28 °C, to an accuracy of ±2 °C. Any change in temperature shall be justified and clearly indicated in the test report.

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

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 (MgCl ₂ · 6 H ₂ O)	9,7 g
Sodium sulfate (Na ₂ SO 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. Report this pre-treatment process in the test report.

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7 Apparatus

7.1 Test flasks

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Biometer flasks of the volume of about 250 ml are appropriate. 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, Annex 4.[6]

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 0,025 N or with 3 ml of KOH 0,5 N.

7.3 Analytical balance

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.