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## Plastics — Test method for determining aerobic biodegradation of plastic materials sunk at the sea water/sandy sediment interface

*Plastiques — Méthode d'essai pour la détermination de la biodégradation aérobie des matières plastiques immergées à l'interface eau de mer/sédiments sableux*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 18830 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

This second/third/... edition cancels and replaces the first/second/... edition (), [clause(s) / subclause(s) / table(s) / figure(s) / annex(es)] of which [has / have] been technically revised.

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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.

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# Plastics — Test method for determining aerobic biodegradation of plastic materials sunk at the sea water/sandy sediment interface

## 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 oxygen demand in a closed respirometer.

Measurement of aerobic biodegradation can also be obtained by monitoring the carbon dioxide evolution, but this is not in the scope of this International Standard. This test method is a simulation of the habitat found in a benthic zone where sunlight reaches the ocean floor (photic zone) that, in marine science is called sublittoral zone.

Plastic products are directly littered or arrive with fresh waters in the pelagic zone (free water). From there, and depending on density, tides, and currents, 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. The determination of biodegradation of plastic materials buried in marine sediment is outside the scope of this International Standard.

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

The following referenced documents are indispensable for the application 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 14851:1999, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer*

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

#### **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, expressed as milligrams of oxygen uptake per milligram or gram of test compound

**3.2 theoretical oxygen demand (ThOD)**  
theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula, expressed as milligrams of oxygen uptake per milligram or gram of test compound

**3.3 total organic carbon (TOC)**  
all the carbon present in organic matter which is dissolved or suspended in water

**3.4 pre-conditioning phase**  
the 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 biological oxygen demand (BOD) and it derives from ISO 14851. The testing medium is based on a solid phase and a liquid phase. The solid phase is 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, 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.

Example of a respirometer suitable for the purpose is given in ISO 14851:1999, Annex C.

The level of biodegradation is determined by comparing the BOD with the theoretical amount (ThOD) and expressed in per cent. 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 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  $\pm 2$  °C. Any change in temperature shall be justified and clearly indicated in the test report.

NOTE Pay attention to the fact that 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 ( $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ )	9,7 g
Sodium Sulfate ( $\text{Na}_2\text{SO}_4$ )	3,7 g
Calcium Chloride ( $\text{CaCl}_2$ )	1 g
Potassium Chloride (KCl)	0,65 g
Sodium Hydrogen Carbonate ( $\text{NaHCO}_3$ )	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 four 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 0,1 % to 2 %.

## 7 Apparatus

**7.1 Closed respirometer** including test vessels (glass flasks) and all other necessary equipment, shall be located in a constant-temperature room or in a thermostat apparatus (e.g. water-bath). For an example see annex A or ISO 14851:1999, Annex C. Stirring can be applied on seawater on condition that it does not disturb the sediment/seawater interface. An example of a stirred apparatus is given in Annex 4 of OECD TG 308: Aerobic and Anaerobic Transformation in Aquatic Sediment Systems [3].

NOTE 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 for measuring total organic carbon (TOC) and dissolved organic carbon (DOC) (see ISO 8245).

### 7.2 Analytical equipment for measuring nitrate and nitrite concentrations

NOTE A qualitative test 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 (usual laboratory equipment)