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Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface — Method by measuring the oxygen demand in closed respirometer

Plastiques — Détermination de la biodégradation aérobie des matières plastiques immergées à l'interface eau de mer/sédiments sableux — Méthode par mesurage de la demande en oxygène dans un respiromètre fermé

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Foreword

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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.*

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 foiling 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.

Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface — Method by measuring the oxygen demand in closed respirometer

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. This is not in the scope of this International Standard but of ISO 19679.

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.

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 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 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

Note 1 to entry: It is 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

Note 1 to entry: It is 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
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 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.

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.

The details of interlaboratory testing based on the test method specified in this International Standard are available in Reference [4].

5 Test environment

Incubation shall take place in the dark or diffused 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 ($MgCl_2 \cdot 6\ H_2O$)	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. Report this pre-treatment procedure in the test report.

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 OECD TG 308, Annex 4.[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 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 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)

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