INTERNATIONAL STANDARD

ISO 22404

First edition
2019-09

Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide

Plastiques — Détermination de la biodégradation aérobie des matériaux non flottants exposés aux sédiments marins — Méthode par analyse du dioxyde de carbone libéré

Reference number
ISO 22404:2019(E)

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Published in Switzerland

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Foreword

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*.

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 and other biodegradable materials are designed to be recoverable 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 (for example, 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. In order to carry out a proper product design, it is important to know whether a plastic material is inherently biodegradable when exposed to marine inocula.

This document provides a test method for calculating and reporting biodegradation level obtained under laboratory conditions using a marine inoculum. The marine inoculum is sediment sampled at the tidal zone. The plastic material is exposed to this environmental matrix and biodegradation is followed by measuring the evolved CO_2.

Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide

1 Scope

This document specifies a laboratory test method to determine the degree and rate of aerobic biodegradation level of plastic materials. This test method can also be applied to other materials.

Biodegradation is determined by measuring the CO_2 evolved by the plastic material when exposed to marine sediments sampled from a sandy tidal zone and kept wet with salt-water under laboratory conditions.

This test method is a simulation under laboratory conditions of the habitat found in sandy tidal zone that, in marine science, is called eulittoral zone.

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

Deviations from the test conditions described in this document are justified in the test report.

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 10210, *Plastics — Methods for the preparation of samples for biodegradation testing of plastic materials*

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
tidal zone
borderline between sea and land that extends from the high tide line, which is rarely inundated with water, to the low tide line, which is typically always covered with water

Note 1 to entry: The tidal zone is frequently a sandy area that is kept constantly damp by the lapping of the waves.

Note 2 to entry: Stony and rocky shorelines also exist.

Note 3 to entry: They are also known as eulittoral zone, midlittoral zone, mediolittoral zone, intertidal zone, foreshore.

3.2
theoretical amount of evolved carbon dioxide
$ThCO_2$
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)* (3.3) and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound

3.3
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 milligrams of the compound.

3.4
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 ms^{-2} for 15 min or by membranes with pores of 0,2 µm to 0,45 µm diameter

3.5
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 the evolved carbon dioxide and derives from ISO 19679. The testing medium is based on a sandy marine sediment laid in the bottom of a closed flask; the sediment is kept wet with natural sea water. The test material is preferably in the form of a powder.

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 ($ThCO_2$) and expressed in per cent. 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 14852:2018, Annex A.

5 Test environment

Incubation shall take place in the dark 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 can be different from real conditions in marine environment.

6 Reagents

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

6.2 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. Before use remove any obvious large material (such as plant material, shells).

NOTE 1 It is possible to obtain sediment from multiple samples collected from different locations to increase microbial variability.

NOTE 2 Seawater and sediment can also be sampled from large, well running public marine aquaria.

Measure the TOC, pH and nitrogen content of the sediment. The total organic carbon content of sediment should be in the range 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 in the test report.

7 Apparatus

7.1 General

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.

Required is usual laboratory equipment, plus the following.

7.2 Test flasks. Biometer flasks of the volume of about 2 l to 4 l are appropriate. The vessels shall be located in a constant-temperature room or in a thermostatic apparatus (such as water-bath). Reactors with higher or lower volumes can be used, if environmental conditions are not affected.

7.3 Container for the CO_2 absorber. A glass beaker to be located in the headspace of the reactor and filled with 100 ml of $Ba(OH)_2$ 0,025 N or with 30 ml of KOH 0,5 N.

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

7.5 pH meter.

8 Procedure

8.1 Test material

The test material should be preferably milled. Preparation of powder from plastic materials shall be done according to ISO 10210. Alternatively introduce the test material in film or sheet form. The sample shall be of known mass and contain sufficient carbon to yield CO_2 that can be adequately measured by the system used. Use a test material concentration of at least 25 mg/100 g of sediment. This mass of the sample should correspond to TOC of about 15 mg/kg. The maximum mass of sample per flask is limited by the oxygen supply to the glass flask. The use of 40 mg to 75 mg of test material per 100 g sediment is recommended. Calculate the TOC from the chemical formula or determine it by a suitable analytical technique (for example, elemental analysis or measurement in accordance with ISO 8245) and calculate the $ThCO_2$.