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Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions in laboratory

Plastiques — Détermination de la biodégradation aérobie des matières plastiques exposées à l'eau de mer en utilisant des conditions accélérées en laboratoire

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ISO copyright office
CP 401 • CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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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 document 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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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.

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Introduction

Plastics have been used in various fields because of their highly durable characteristics. Plastics are also used intentionally in the environment or in applications where unintentional loss to the environment is unavoidable. The degree of biodegradation of plastics in natural environment is of interest in order to better characterize the behaviour of plastics in these very particular environments. Thus, the test methods to measure the degree and rate of biodegradation are of major interest to obtain an indication of the potential biodegradability of plastic materials when exposed to different marine habitats.

The biodegradability of plastics is defined in laboratory tests, for example ISO 18830, ISO 19679, ISO 23977-1 and ISO 23977-2, but these test methods in a laboratory basically use only seawater and sediment.

Since existing test methods are not intended to shorten the test period, the test period often extends to one year and it is sometimes difficult to assess the degree of biodegradability of the sample in a short period of time.

This document provides an accelerated test method for determining the biodegradation level of plastics exposed to seawater in laboratory in shorter time by increasing the number of microorganisms in seawater and enriching the flora, by adding nutrients, pre-treatment by sediment, and condensation of seawater. The degree of biodegradation is calculated based on measurement of the oxygen (O_2) demand in a closed respirometer or carbon dioxide (CO_2) evolution.

Test material is taken up by microorganisms, metabolized, and the contained carbon finally mineralized to CO_2. Part of the carbon originating from the test material is temporarily converted to biomass. The biomass cannot be detected unless the bacteria die and undergo self-digestion. It takes a longer period of time than the conversion to biomass and it may require a long test period to complete the mineralization of the test material. On the other hand, since biomass components are produced by the microbial activity, the total biodegradation considered by carbon flow can be estimated by the sum of the degree of biodegradation based on O_2 and/or CO_2 analysis and the degree of conversion to biomass. Therefore, for the purpose of promoting the development of biodegradable plastics, this document also provides a method for measuring the biomass components produced by metabolizing the test material before it is completely mineralized to CO_2, that enables to evaluate the potential biodegradability considered by carbon flow in much shorter period of time than full mineralization.

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Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions in laboratory

1 Scope

This document describes a laboratory test method to determine the aerobic biodegradation of plastic materials exposed to seawater using accelerated conditions. It describes the general requirements of the apparatus and the procedures for using this test method.

This test method is designed to give an early indication of the potential biodegradability of plastic materials in marine environment.

For the purpose of promoting the development of biodegradable plastics, this document also provides a method for measuring the biomass components produced by metabolizing the test material before it is completely mineralized to CO_2, that enables to evaluate the potential biodegradability considered by carbon flow by the sum of the degree of biodegradation based on O_2 or CO_2 analysis and the degree of conversion to biomass in much shorter period of time than full mineralization.

This method is not suitable to assess the degree of disintegration of plastic materials caused by abiotic factors like heat or UV radiation.

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 472, *Plastics — Vocabulary*

ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

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

ISO 10523, *Water quality — Determination of pH*

ISO 11261, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method*

ISO 23977-1, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide*

ASTM D1141-98, *Standard Practice for Preparation of Substitute Ocean Water*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 472 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

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3.1
carbon flow
series of compounds produced from carbon derived from plastics during biodegradation

Note 1 to entry: The series of compounds includes polymers with reduced molecular weight, water-soluble oligomers, compounds in the process of biochemical metabolism, organic matter that makes up bacterial cells, and carbon dioxide that is produced when plastics are completely biodegraded.

3.2
^{1}H NMR
proton NMR
nuclear magnetic resonance using proton as the observed nucleus

3.3
pelagic seawater
pelagic zone
water body above the seafloor

Note 1 to entry: It is also referred to as the open water or the water column.

Note 2 to entry: The surface of the pelagic zone is moved by wind-driven waves, is in contact with the atmosphere and exposed to sunlight. With increasing depth pressure increases, temperature decreases, and light and surface wave energy are attenuated.

[SOURCE: ISO 22766:2020, 3.4]

3.4
lag phase
time from the start of a test until adaptation and/or selection of the degrading microorganisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the *maximum level of biodegradation* (3.10)

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.8]

3.5
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.

[SOURCE: ISO 18830:2016, 3.1]

3.6
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.

[SOURCE: ISO 18830:2016, 3.2]

3.7
total organic carbon
TOC
amount of carbon bound in an organic compound

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

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[SOURCE: ISO 17556:2019, 3.14]

3.8
dissolved organic carbon
DOC
part of the organic carbon in water which cannot be removed by specified phase separation

Note 1 to entry: Phase separation can be achieved for example by centrifugation at 40 000 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

[SOURCE: ISO 14852:2021, 3.7]

3.9
theoretical amount of evolved carbon dioxide
$ThCO_2$
maximum theoretical amount of carbon dioxide evolved after completely oxidizing a chemical compound, calculated from the molecular formula

[SOURCE: ISO 23977-1:2020, 3.3, modified – Note 1 to entry was removed.]

3.10
biodegradation phase
time from the end of the *lag phase* (3.4) of a test until the plateau phase has been reached

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.10]

3.11
maximum level of biodegradation
degree of biodegradation of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

Note 1 to entry: It is measured in per cent.

[SOURCE: ISO 14852:2021, 3.9]

3.12
plateau phase
time from the end of the *biodegradation phase* (3.10) until the end of a test

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:2021, 3.11]

4 Principle

This document describes a test method for determination of the aerobic biodegradation of plastic materials exposed in seawater using accelerated conditions in laboratory. The biodegradation is determined by measuring the oxygen demand in a closed respirometer or by measuring the CO_2 evolved during mineralization of the plastic materials by microorganisms in the seawater.

The biochemical oxygen demand (BOD) is determined by consumption of oxygen, 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 calculated by dividing the BOD by the theoretical oxygen demand (ThOD) and is 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.

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