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Plastics — Marine biodegradation testing — Preparation of optimized intertidal seawater and sediment

Plastiques — Essais de biodégradation en milieu marin — Préparation d'eau de mer et de sédiments intertidaux optimisés

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Foreword

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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.

Introduction

The assessment of the degree of biodegradation of plastics in marine habitats is one effective measure to understand and evaluate the impact of reuse, recycling and environmental pollution of plastics. The biodegradation of plastics is the process, in which plastics are decomposed by heterotrophic microorganisms, such as bacteria and fungi, through enzymatic hydrolysis and subsequent metabolization. Marine biodegradation proceeds mainly in microbial consortia that form at the interface between seawater and plastics. This is because marine microorganisms live aerobically within biofilms at the interface between the liquid phase of seawater and solid phases such as gravel and shells.

The diversity of microbial consortia in the marine environment is high, depending on their natural environmental conditions. The species and number of microorganisms vary depending on the climate, ocean currents, tides, and topography. Considering the diverse habitats of these microorganisms, three types of biodegradation assessment methods have been developed:

— one-phase systems consisting of seawater or sediment and

— two-phase systems consisting of seawater and seafloor sediments.

However, due to the diversity of microorganisms even a biodegradable material such as cellulose, which is used as a reference material, gave biodegradation results that ranged from 0 to 100 percent in ring tests of these test methods. From the perspective of biodegradable plastic specification, it is thus necessary to optimize the preparation of natural inoculum for the biodegradation tests to avoid those fluctuations in experimental outcomes.

In order to reduce the impact of seasonal and regional variation in the marine inoculum composition, this document describes a method for preparing seawater and seafloor sediments. The prepared seawater and sediment can be used for the test methods defined in ISO 19679, ISO 18830, ISO 22404, ISO 23977-1, ISO 23977-2 and ISO 23832.

Prepared seawater for biodegradation tests is obtained by rinsing seafloor sediments with seawater. Sand and gravel mixtures with particle sizes from 250 µm to 2 mm are used as sediments to provide pore water flow, oxygen supply, seawater filtration and biofilm growth. Through the preparation of defined compositions of seawater and sediments in marine tests, the number of microorganisms and aerobic conditions are stabilized, and reproducibility and comparability of biodegradation experiments (including curves, lag time, etc.) are improved.

This document specifies methods for preparing seawater and sediments in the intertidal zone for estimating the aerobic biodegradation of plastics in pelagic to coastal marine environments.

Plastics — Marine biodegradation testing — Preparation of optimized intertidal seawater and sediment

1 Scope

This document specifies procedures for preparing seawater and sediments used in test methods to assess the biodegradation of plastic materials in the marine environment. The screened sediment and sediment-rinsed seawater are prepared to sustain aerobic testing at laboratory scale. The described method is designed to separate sediment-rinsed seawater and sand-gravel sediments from intertidal sediments by wet filtration and seawater flotation. This document does not include steps to enhance the biodegradation of plastic materials by concentrating the natural seawater, adding nutrients to the seawater, and pre-culturing the inoculum.

The methods described in this document are intended to be used in addition to issued ISO standard test methods for evaluating the biodegradation and disintegration of plastic materials. The applicable evaluation test methods are ISO 18830, ISO 19679, ISO 22404, ISO 23977-1, ISO 23977-2 and ISO 23832.

NOTE The conditions described in this document do not always correspond to the optimum conditions for maximum biodegradation. This is a method of preparing test sediments from coastal seafloor sediments, not a method of preparing sediments from deep-sea seafloors.

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 18830, *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*

ISO 19679, *Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface — Method by analysis of evolved carbon dioxide*

ISO 22404, *Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide*

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

ISO 23977-2, *Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer*

ISO 23832, *Plastics — Test methods for determination of degradation rate and disintegration degree of plastic materials exposed to marine environmental matrices under laboratory conditions*

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 https://www.electropedia.org/

3.1
intertidal 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.

[SOURCE: ISO 22404:2019, 3.1]

3.2
biofilm
microbial cells and their metabolites, such as polysaccharides, proteins, lipids and nucleic acids, firmly attached to the material surface of the product in water, and stained with crystal violet

[SOURCE: ISO 4768:2023, 3.1]

3.3
biodegradation
degradation (3.4) caused by biological activity, especially by enzymatic action, leading to a significant change in the chemical structure of a material

[SOURCE: ISO 472:2013, 2.1680]

3.4
degradation
irreversible process leading to a significant change in the structure of a material, typically characterized by a change of properties (e.g. integrity, molecular mass or structure, mechanical strength) and/or by fragmentation, affected by environmental conditions, proceeding over a period of time and comprising one or more steps

[SOURCE: ISO 472:2013, 2.262]

3.5
disintegration
physical breakdown of a material into very small fragments

[SOURCE: ISO 472:2013, 2.1757]

3.6
theoretical amount of evolved carbon dioxide
$ThCO_2$
maximum carbon dioxide evolved after completely oxidising a chemical compound, calculated from the molecular formula or from determination of *total organic carbon (TOC)* (3.7)

[SOURCE: ISO 19679:2020, 3.1, modified — “theoretical amount of” removed after “maximum”.]

3.7
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.

[SOURCE: ISO 17556:2019, 3.14]

3.8
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

[SOURCE: ISO 472:2013, 2.1723]

3.9
total dry solids
amount of solids obtained by taking a known volume of test material or inoculum and drying at about 105 °C to constant mass

[SOURCE: ISO 13975:2019, 3.5]

3.10
volatile solids
amount of solids obtained by subtracting the residues of a known volume of test material or inoculum after incineration at about 550 °C from the *total dry solids* (3.9) content of the same sample

Note 1 to entry: The volatile solids content is an indication of the amount of organic matter present.

[SOURCE: ISO 17088:2021, 3.9]

4 Principle

Biodegradable plastics in seawater are primarily degraded into water and inorganic carbon dioxide, and partially assimilated into biomass by heterotrophic microorganisms in the marine food chain. In order to evaluate aerobic biodegradation on a laboratory scale, the culture conditions such as nutrients, pH and microbial species should be specified in the actual seawater and seafloor sediments used. Furthermore, marine microorganisms survive aerobically as microbial communities in biofilms that form at the interface between the liquid phase of seawater and solid phases, such as gravel, shells, and plastic films. Therefore, marine biodegradation is dependent on the marine ecological environment, and the preparation methods of seawater and sediment for marine biodegradation testing also need to be identified.

The pH of seawater is approximately 8,1, while the nutrient levels, biomass, microorganism abundance and diversity are influenced by habitat and seasonal variation.

Interlaboratory tests according to ISO 19679 and ISO 18830 were conducted in nine laboratories in seven countries, as shown in Annex I. At the end of the test, the average carbon dioxide production per gram of wet sediment was 2,1 mg, with values ranging from 0,63 mg to 4,88 mg. The biodegradation value of reference filter paper ranged from 5 % to 160 %, with an average value of 87 % and a coefficient of variation of 36 %. Similarly, an interlaboratory test was also conducted to improve the OECD 306 screening test, as the biodegradation outcome varies depending on the abundance and composition of the microbial community[8].

In particular, the number of viable microorganisms in coastal areas is thousands of times higher than in pelagic areas. On the other hand, sediments maintain aerobic conditions due to characteristics, such as oxygen-saturated water flow, fine particle filtration, and pore water circulation. Sediments also serve as a source of organic carbon, which is necessary for microbial growth, and source of calcium carbonate, which helps buffer the pH of seawater.

This method of preparation of rinsed seawater and refined sediments significantly improves these values for plastic test materials listed in Annex D and H.

In this preparation method, sediments in the sand-gravel area, including shells and corals, are selected from the subseafloor sediments in the intertidal zone of the coastal area by sorting based on the particle size of the object. Seawater for biodegradability testing is collected by washing the seafloor sediments and sand-gravel surface overgrown with biofilms using seawater.

Seafloor sediments are wet filtered using a 2 mm sieve in a container filled with seawater. This sieve is used for soil identification and classification according to ISO 14688-1, removing gravel and collecting clay, silt, and sand based on particle size. Wet filtration separates the seafloor sediments into two layers: a lower layer consisting of a sludge-like sediment including clay, silt, sand, gravel, benthic organisms, and eggs, and an upper layer comprising a seawater suspension containing floating pieces of biofilm and microorganisms. Larger aggregated floating particles are removed from the seawater using a filter paper having a pore size of about 20 µm. This is to obtain filtered seawater containing microorganisms. The sludge-like sediments are refined into sand-gravel sediments by flotation with seawater. By repeating flotation, as shown in Annex A and B, more than 5 times, sediments with a particle size of 250 µm or more are prepared and can be used for biodegradation testing. This sediment preparation method produces larger sediment particles than plastic powder samples prepared according to ISO 10210.

Compared to unprepared pelagic seawater, this preparation method provides seawater with potentially higher microbial diversity and cell count, which can lead to increased biodegradation rates. The prepared seawater and sediment are effective in emulating the biodegradation of plastic materials in marine environments based on BOD and carbon dioxide evolved in laboratory-scale testing.

5 Apparatus

5.1 Sieves, with 2 mm~3 mm opening and 250 µm or 300 µm mesh for filtering sand-gravel by wet filtration method.

5.2 Bowls, two or more 15 l~20 l bowls (e.g. stainless steel) for the kitchen to prepare the sediments by flotation and wet filtration of seawater and sediments.

5.3 Shovel, for collecting top sediment (the layer from surface till about 20 cm depth).

NOTE The type is a pointed digging shovel or gardening shovel about 1 m long.

5.4 Weight scale, capable of weighing 20 kg of seawater or sediment.

5.5 pH Meter, used for measurement of the pH of the marine test mixture. It shall be accurate to 0,1 pH-units or better.

6 Procedure

6.1 General

In the intertidal zone, microorganisms form biofilms at the interface between sediments and seawater. These biofilms exist in aerobic conditions. To collect biofilm-covered sediment and seawater rich in microorganisms, the collected seafloor sediment is passed through a sieve with a pore size of 2 mm in the seawater to remove gravel and seaweed (wet filtration). The filtrate is separated into a suspension and a sludge-like sediment. The suspension separated by decantation is suction-filtered using a filter paper with a pore size of 20 µm to prepare seawater for testing. The sludge-like sediment is washed away by seawater flotation and becomes sand-gravel sediments covered with biofilm. These processes include the removal of benthic organic matter.

Purified seawater and sediment shall be pre-incubated or stored according to the biodegradation or disintegration test methods specified in ISO 18830, ISO 19679, ISO 22404, ISO 23977-1, ISO 23977-2, and ISO 23832.

The purification and sieving steps can be performed outdoors at the sampling point or indoors in the laboratory after transporting the samples taken at sea. In this case, artificial seawater can be used. Artificial seawater formulation shall be in accordance with ISO 18830 or ISO 19679.

Collect top sediment layers from the surface to a depth of about 20 cm, suitable for lab-scale biodegradation tests.