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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

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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 documents 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.

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 $ThCO_2$.

The form and shape of the test material may influence its biodegradation speed. Similar shapes and thicknesses should preferably be used if different kinds of plastic materials are to be compared. A particle size distribution with the maximum at 250 µm diameter is recommended.

The test material may also be introduced as a film. When the test material in form of film is buried in the sediment it could limit the gas exchange between the water body and the sediment, promoting the formation of anaerobic zones under the test material. In order to reduce this effect, it is possible to perforate the film sample homogeneously over the entire surface.

8.2 Reference material

Use microcrystalline cellulose or ashless cellulose filters as a reference material[1]. If possible, the TOC, form and size should be comparable to that of the test material. As a negative control, a non-biodegradable polymer (e.g. polyethylene) in the same form as the test material should be used.

8.3 Preparation of the sediment

Filter the sediment in a funnel with a coarse filter paper to eliminate excess seawater. Sediment is ready for testing when dripping of sea water is ended. Sediment after filtering is named “wet sediment” hereafter. Nitrogen (such as NH_4Cl or $NaNO_3$) can be added to the sediment if this is considered as a factor limiting biodegradation. It is suggested to add 0,1 g of N per g of TOC of test item added to the test system. These additions shall be reported in the test report.

It is recommended that the ratio between organic carbon and nitrogen (C/N ratio) of the test mixture is optimized so as to ensure optimum biodegradation conditions. The C/N ratio for the test mixture should preferably be between 10 and 40.

8.4 Test setup

Provide a number of flasks, so that the test includes at least the following:

a) three flasks for the test material (symbol F_T);

b) three flasks for the blank (symbol F_B);

c) three flasks for reference material (symbol F_C).

In addition, it is recommended to add:

d) three flasks for negative control (symbol F_N).

Two flasks for test material, blank, reference material and negative control may be used instead of three for screening purposes.

8.5 Pre-conditioning phase

In a typical case, use a test flask with a volume of 3 L. Laid down equal amount (between 200 g and 600 g, typically 400 g) of the wet sediment on the bottom of each flask to form a homogenous layer. Do not press or compact the sediment.

Add carbon dioxide absorber to the absorber compartments of the test flask in a typical case 30 ml of KOH 0,5 N or 100 ml of $Ba(OH)_2$ 0,025 N. Place the flasks in a constant-temperature environment and allow all vessels to reach the desired temperature. Take the necessary readings and monitor the CO_2 evolution.

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[1] Microcrystalline Cellulose "Avicel" produced by Merck or Laboratory filter paper Whatman n°42 have been found satisfactory for this purpose. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

This phase is carried out in order to verify that the endogenous respiration is similar in the different vessels and also to obtain a preliminary oxidation of excess organic matter, in order to start the test with a lower endogenous respiration.

This phase is generally protracted for a week but is possible to extend this time if a high amount of CO_2 evolved is measured.

In case the CO_2 evolution of a vessel is different (i.e. more than 30 % of the average CO_2 evolution of all the vessels) reject the diverging vessel or in case of multiple anomalies, start again using new sediment.

Different volumes and amounts can be used if environmental conditions are not affected.

Different amount of sediment can be used if reactors with different volumes are used, on condition that the depth of sediment layer on the bottom is comprised in a range varying from about 2 cm (preferably with fine sediment) to about 4 cm (preferably with coarse sediments).

8.6 Start of the test

Open the flasks. Add the milled test material. Mix thoroughly the sediment with the material and finally form a homogenous layer. Do not press or compact the sediment.

When the material is tested as a buried film, remove part of the sediment, place the film and cover it with the withdrawn sediment forming a homogenous layer.

Mass of samples (test and reference material) should be at least about 100 mg each when using a flask with a volume of 3 L corresponding to an initial test item concentration specified in 8.1.

Repeat the procedure for the reference material and the material for the negative control to the respective flasks. Record the mass of the sediment, the sample introduced in each vessel.

Nutrients may be supplemented as needed to support microbial diversity and to maintain the capacity to biodegrade the test material. The need and timing of additional nutrients or other appropriate measures may be judged by observation of the temporal course of the biodegradation of the reference substance cellulose.

Any addition and the applied method shall be reported in the test report.

8.7 Carbon dioxide measurement

8.7.1 The CO_2 reacts with $Ba(OH)_2$ and is precipitated as barium carbonate ($BaCO_3$). The amount of CO_2 produced is determined by titrating the remaining barium hydroxide with 0,05 N hydrochloric acid to a phenolphthalein end-point or by automatic titrator. Because of the static incubation, the barium carbonate builds up on the surface of the liquid and shall be broken up periodically by shaking the container gently to ensure continued absorption of the evolved CO_2. This problem can be avoided by using KOH instead of $Ba(OH)_2$, which does not form a precipitate.

NOTE A discussion on the use of KOH in place of $Ba(OH)_2$ is reported in Modelli et al[4].

8.7.2 The containers for the CO_2 absorber shall be removed and titrated before their capacity is exceeded. The period of time will vary with sediments and test materials and increases slowly as the carbon content of the sediment is reduced (a recommended frequency of every 3 to 4 days for the first 2 to 3 weeks and every 1 to 3 weeks thereafter). At the time of removal of the containers, the reactor should be allowed to sit open so that the air is refreshed before replacing 100 ml of fresh barium hydroxide and resealing the reactor. The reactors should remain open approximately 15 min.

If different volumes and amounts are used, then the volume of the CO_2 absorber should be proportional to the used volumes. For example, if 250 ml-flasks are used, 10 ml of fresh barium hydroxide can be used.

8.7.3 The carbon dioxide evolution rate may reach a plateau when all of the accessible carbon has been oxidized. The test may be terminated at this point or earlier, at the discretion of the user. If possible, the residual test material may be extracted from the sediment with an appropriate method and quantified (optional).

NOTE The evolved CO_2 can be quantitatively measured also using other suitable methods such as those based on infrared CO_2-analysers or those based on TOC analysers equipped with an infrared photometer.

8.8 End of the test

When a constant level of CO_2 evolution is attained (plateau phase reached) and no further biodegradation is expected, the test is considered to be completed. The maximum test period is 24 months. In the case of long test durations, special attention shall be paid to the technical system (e.g. tightness of the test vessels and connections). Any special measures taken, for example, to ensure microbial diversity or to provide sufficient nutrients shall be detailed in the test report.

9 Calculation and expression of results

9.1 Calculation

9.1.1 Amount of CO_2 produced

9.1.1.1 General

The first step in calculating the amount of CO_2 produced is to correct the test material reactors for endogenous CO_2 production. The control reactor serves as a blank to correct for CO_2 which may be produced through endogenous respiration of the microorganisms. The amount of CO_2 produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank containers. The next step is to convert ml HCl titrated into mg of CO_2 produced.

9.1.1.2 $Ba(OH)_2$ used as CO_2 absorber

When CO_2 enters the absorber containers, it reacts in the following manner:

$$\mathrm{Ba(OH)_2 + CO_2 \rightarrow BaCO_3 s + H_2O} \tag{1}$$

The $BaCO_3$ formed is insoluble and precipitates. The amount of $Ba(OH)_2$ remaining in solution is determined by titration of the 10 ml with HCl according to Formula (2):

$$\mathrm{Ba(OH)_2 + 2\,HCl \rightarrow BaCl_2 + 2H_2O} \tag{2}$$

From Formulae (1) and (2) it can be seen that 1 mmol of CO_2 is produced for every 2 mmol of HCl titrated. This means that the number of mmol of CO_2 produced is as shown in Formula (3):

$$\mathrm{mmol\,CO_2} = \frac{\mathrm{mmol\,HCI}}{2} \tag{3}$$

The normality of HCl used is 0,05 N. Substituting for mmol gives Formula (4):

$$\text{mmol CO}_2=\frac{(0{,}05\,N)\times(\text{ml of HCl})}{2} \tag{4}$$

To convert to mg CO_2, the value shall be multiplied by the molecular weight of CO_2 which is 44 mg/mmol, as shown in Formula (5):

$$\text{mg CO}_2=\frac{((0{,}05)\times\text{ml titrated})}{2}\times 44=1{,}1\text{ ml of HCI titrated} \tag{5}$$

Thus, to convert ml of HCl to mg CO_2, the former is multiplied by 1,1.

9.1.1.3 KOH used as CO_2 absorber

The evolved CO_2 will react with KOH in the following manner:

a) $2KOH + CO_2 \rightarrow K_2CO_3 + H_2O$

K_2CO_3, the product of reaction (a) is soluble and does not precipitate.

The fresh KOH solution, where no CO_2 has been absorbed, can be titrated with HCl as:

b) $KOH + HCl \rightarrow KCl + H_2O$, at pH 7

The KOH solutions used as CO_2 absorbers will have both unreacted KOH and K_2CO_3 as per (a).

During titration both chemical species will react with HCl, as follows:

c) $KOH + HCl \rightarrow KCl + H_2O$, at pH 7

d) $K_2CO_3 + HCl \rightarrow KHCO_3 + KCl$, at pH 8,5

The pH shifts of reactions (b) and (c) are superimposed and cannot be distinguished. Only a single end point in the range of pH between 7 and 8, corresponding to the two reactions, can be identified by using a suitable indicator.

The adsorbed CO_2 can be determined by subtracting from the H^+ equivalents needed to neutralize the original KOH solution and the H^+ equivalents needed to neutralize the reactions (c) and (d). In practice:

$$\text{mmol CO}_2 = [\text{ml HCl consumed (b)} - \text{ml HCl consumed in (c) + (d) end point}] \cdot \text{N HCl} \tag{6}$$

where N is the normality of the HCl solution.

If an end point titrator is available the mmol of CO_2 can be determined, without using an indicator, with a further reaction. A further addition of HCl makes HCl react with $KHCO_3$, produced with reaction (d):

e) $KHCO_3 + HCl \rightarrow H_2CO_3 + KCl$, at pH4

The number of equivalent consumed in reaction (e), and therefore in reaction (d), corresponds to the K_2CO_3 produced during reaction (a) that in turn corresponds to the absorbed CO_2.

Consequently 1 mole of $KHCO_3$ corresponds to 1 mole of CO_2 reacted in reaction (a):

$$\text{mmol } CO_2 = \text{mmol HCl consumed in (e) end point} \tag{7}$$

Therefore:

$$\text{mmol } CO_2 = \text{ml HCl consumed in (e)} \cdot \text{N HCl} \tag{8}$$

where N is the normality of the HCl solution.

The amount of CO_2 expressed in milligrams is finally obtained using Formula (9):

$$\text{mg } CO_2 = \text{mmol } CO_2 \cdot 44 \tag{9}$$

9.1.2 Percentage of biodegradation

The percentage of biodegradation is the ratio between the evolved CO_2 and theoretical CO_2 ($ThCO_2$). The $ThCO_2$ is shown in Formula (10):

$$ThCO_2 = \text{specimen (mg)} \times \text{TOC(\%)} \times \frac{44}{12} \tag{10}$$

where

TOC (%) is the TOC of the plastic material (or reference material) divided by 100;

44 is the molecular weight of CO_2;

12 is the molecular weight of C.

Therefore:

$$\text{\%Biodegradation} = \frac{\text{mg } CO_2 \text{ produced}}{\text{Th } CO_2} \times 100 \tag{11}$$

9.2 Expression and interpretation of results

Compile a table of the CO_2 values measured and the percentages of biodegradation for each measurement interval and each test flask. For each vessel, plot an evolved CO_2 cumulative curve and a biodegradation curve in per cent as a function of time.

A curve of mean biodegradation values may be plotted.

The maximum level of biodegradation determined as the mean value of the plateau phase of the biodegradation curve or the highest value, e.g. when the curve decreases or, further on, slowly increases in the plateau phase, characterizes the degree of biodegradation of the test material.

The wettability and the shape of the test material may influence the result obtained, and hence the test procedure may be limited to comparing plastic materials of similar chemical structure.

Information on the toxicity of the test material may be useful in the interpretation of test results showing a low biodegradability.