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

Plastiques — Détermination de la biodégradation aérobie des matières plastiques non-flottantes à l'interface eau de mer/sédiments — 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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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 fouling plastics 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/sediment interface — Method by analysis of evolved carbon dioxide

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 evolved carbon dioxide.

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.

Measurement of aerobic biodegradation can also be obtained by monitoring the oxygen consumption, as described in ISO 18830.

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 14852:1999, *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide*

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
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)

Note 1 to entry: It is expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.

3.2
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.

3.3
dissolved organic carbon
DOC
that 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

3.4
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 evolved carbon dioxide and derives from ISO 14852. The testing medium is based on a solid phase and a liquid phase. The solid phase is a 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.

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 percentage. 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 14852:1999, Annex A.

The details of interlaboratory testing based on the test method specified in this International Standard are available in Reference [5].

5 Test environment

Incubation shall take place in the dark or in diffuse 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 process in the test report.

7 Apparatus

7.1 Test flasks

Biometer flasks of the volume of about 250 ml are appropriate. The vessels shall be located in a constant-temperature room or in a thermostatic apparatus (e.g. water-bath). Stirring can be applied on seawater on condition that it does not disturb the sediment/seawater interface.

NOTE A suitable apparatus is shown in Figure A.1. An example of a stirred apparatus is given in OECD TG 308, Annex 4.[6]

7.2 Container for the CO_2 absorber

A glass beaker to be located in the headspace of the reactor and filled with 10 ml of $Ba(OH)_2$ 0,025 N or with 3 ml of KOH 0,5 N.

7.3 Analytical balance

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

7.4 pH meter

8 Procedure

8.1 Test material

The test material should be in film or sheet form. Cut samples of the test material in the shape of a disk. Disks shall have a smaller diameter than the glass flasks, so that the disks can be easily laid on the bottom of the glass flask.

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 100 mg/l of seawater plus sediment. This mass of the sample should correspond to TOC of about 60 mg/l. The maximum mass of sample per flask is limited by the oxygen supply to the glass flask. The use of 150 mg to 300 mg of test material per litre seawater plus sediment is recommended.

Calculate the TOC from the chemical formula or determine it by a suitable analytical technique (e.g. 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 biodegradability. Similar shapes and thicknesses should preferably be used if different kinds of plastic materials are to be compared.

NOTE 1 The test material may also be introduced as powder. However, this can be critical, as practical experience has shown that it is difficult to keep a powder settled at the sediment/seawater interface without special measures. Refer to ISO 10210 for preparation of powder from plastic materials.

NOTE 2 When the test material in form of film is laid down on the surface of 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 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 can optionally 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.

8.4 Test setup

Provide several 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 possible to add three more flasks for negative control (symbol F_N), if required.

NOTE 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 250 ml. Lay down 30 g of the wet sediment on the bottom of the flask. Carefully pour 70 ml of natural or artificial seawater. The test should be performed with a water/sediment volume ratio between 3:1 and 5:1 and a sediment layer of about 0,3 cm to 0,5 cm, depending on the granulometry of the sediment.

NOTE When using very coarse-grained sediment, the layer may be increased up to 1,5 cm.

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1) Laboratory filter paper Whatman n° 42 has been found satisfactory for this purpose and is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Add carbon dioxide absorber to the absorber compartments of the test flask in a typical case 3 ml of KOH 0,5 N or 10 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.

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. The inoculum can be gently stirred in order to accelerate the biodegradation of excess organic matter.

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, reject the diverging vessel or in case of multiple anomalies, start again using new sediment.

8.6 Start of the test

Dunk the plastic film sample, cut as described in 8.1, on the sediment of each vessel. Mass of samples (test and reference material) should be about 20 mg each when using a flask with a volume of 250 ml corresponding to an initial test item concentration specified in 8.1. In order to ensure a homogeneous contact between sample and sediment, it is recommended to cover the sample with a suitable cover slip. The cover slips shall be introduced in blank vessels also, for assuring similar conditions.

NOTE A suitable cover slip can be made using a common non-biodegradable vinyl-coated fibreglass mosquito net with a fibre diameter of about 280 µm and a 1,8 mm × 1,6 mm mesh.

For an example, see Annex A.

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 and the volume of seawater 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 Reference [5].

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 two to three 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 10 ml of fresh barium hydroxide and resealing the reactor. The reactors should remain open approximately 15 min.

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 or on gravimetric analysis.

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 e.g. to ensure microbial diversity or to provide sufficient nutrients shall be detailed in the test report. On the last day of the test, measure the pH, acidify all the bottles with 1 ml of concentrated hydrochloric acid in order to decompose the carbonates and bicarbonates, continue the test for 24 h and finally measure the amount of carbon dioxide evolved in each of the series of flasks.

9 Calculation and expression of results

9.1 Calculation

9.1.1 Amount of CO_2 produced

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 millilitres of HCl titrated into milligrams of CO_2 produced.

9.1.1.1 $Ba(OH)_2$ used as CO_2 absorber

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

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 \downarrow + H_2O \quad (1)$$

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

$$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O \quad (2)$$

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 derived using Formula (3):

$$\text{mmol } CO_2 = \frac{\text{mmol HCl}}{2} \quad (3)$$

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

$$\text{mmol } CO_2 = \frac{(0,05\,\text{N}) \times (\text{ml of HCl})}{2} \quad (4)$$

To convert to mg CO_2, the value shall be multiplied by the molecular weight of CO_2 which is 44:

$$\text{mg } CO_2 = \frac{[(0,05) \times \text{ml titrated}]}{2} \times 44 = 1,1 \text{ ml of HCl titrated} \quad (5)$$

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

9.1.1.2 KOH used as CO_2 absorber

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

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O \quad (6)$$

K_2CO_3, the product of Formula (6), is soluble and does not precipitate.

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

$$KOH + HCl \rightarrow KCl + H_2O, \text{ at pH 7} \quad (7)$$

The KOH solutions used as CO_2 absorbers will have both unreacted KOH and K_2CO_3 as per Formula (6).

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

$$KOH + HCl \rightarrow KCl + H_2O, \text{ at pH 7} \quad (8)$$

$$K_2CO_3 + HCl \rightarrow KHCO_3 + KCl, \text{ at pH 8,5} \quad (9)$$

The pH shifts in Formulae (6) and (7) 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 represented by Formulae (8) and (9). In practice:

mmol CO_2 = {ml HCl consumed [Formula (7)] – ml HCl consumed in Formula (8) + Formula (9) end point} × N HCl

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 [Formula (9)]:

$$KHCO_3 + HCl \rightarrow H_2CO_3 + KCl, \text{ at pH } 4 \quad (10)$$

The number of equivalent consumed in Formula (10), and therefore in Formula (9), corresponds to the K_2CO_3 produced during Formula (6), that in turn corresponds to the absorbed CO_2.

Consequently, 1 mole of $KHCO_3$ corresponds to 1 mole of CO_2 reacted in Formula (6):

mmol CO_2 = mmol HCl consumed in Formula (10) end point

Therefore:

mmol CO_2 = ml HCl consumed in Formula (10) × N HCl

where N is the normality of the HCl solution.

The amount of CO_2 expressed in milligrams is finally obtained as follows:

mg CO_2 = mmol CO_2 × 44

9.1.2 Percentage of biodegradation

The percentage of biodegradation is the ration between the evolved CO_2 and theoretical CO_2 ($ThCO_2$). The $ThCO_2$ is:

$$ThCO_2 = \text{specimen (mg)} \times \text{TOC(\%)} \times \frac{44}{12} \quad (11)$$

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:

$$\%\,\text{Biodegradation} = \frac{\text{mg } CO_2 \text{ produced}}{ThCO_2} \times 100 \quad (12)$$

9.2 Visual inspection

At the end of the test, check the condition of the samples. If still present, samples can be retrieved for mass determination, other analysis, and photographs.

9.3 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 percentage 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.

10 Validity of results

The test is considered valid, if

a) the degree of biodegradation of the reference material (F_C) is >60 % after 180 days,

b) the evolved CO_2 of the blank F_B at the end of the test does not exceed 3,5 mg CO_2/g wet sediment (see 8.3) after 6 months,

NOTE This value has been determined in an interlaboratory test.

c) the amount of CO_2 evolved from the three blank F_B are within 20 % of the mean at the plateau phase or at the end of the test,

d) the difference between the percentage biodegradation of the reference material in the different vessels is less than 20 % of the mean at the end of the test.

If a negative control (flasks F_N) has been performed, no significant amount of evolved CO_2 shall be observed.

If these criteria are not fulfilled, repeat the test using another sediment.

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard, i.e. ISO 19679:2016;

b) all information necessary to identify the test and reference materials, including their TOC, $ThCO_2$, chemical;

c) the main test parameters, including test volume, test medium used, incubation temperature and final pH;

d) the source and amount of the marine sediment used;

e) the analytical techniques used, including the principle of the respirometer and the TOC;

f) all the test results obtained for the test and reference materials (in tabular and graphical form), including the evolved CO_2, the percentage biodegradation values;

g) the duration of the lag phase, biodegradation phase and maximum level of degradation, as well as the total test duration; and, optionally, if run or determined, the negative control F_N;

h) any other relevant data (e.g. result of the visual final inspection and analysis of final samples, if still retrievable; photos of the final samples);

i) details of the methods used during the test period in order to support microbial diversity or to avoid nutrient deficiency.

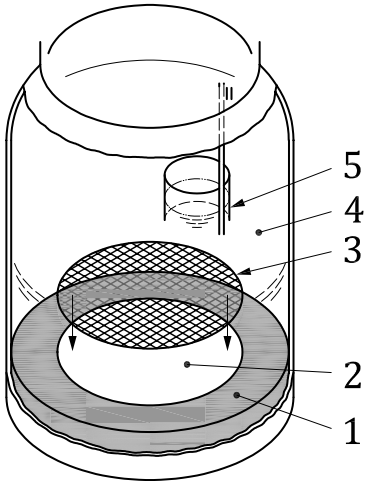
Annex A
(informative)

Example of respirometric system based on CO_2 measurement

The measurement of the evolved CO_2 can be obtained by trapping the evolved CO_2 in a closed system and then by quantifying it by suitable titration systems. Microorganisms in the vessel consume the oxygen and form CO_2. This is absorbed by a CO_2 absorber (generally NaOH) that can then be titrated to determine the amount of the absorbed CO_2.

In a typical case, a 250 ml vessel is used. The sediment occupies about 20 ml, the seawater 70 ml and the headspace 160 ml. The O_2 present in air at 1 atm and 28 °C and a relative humidity of 100 % is about 0,274 261 mg/ml. This means that the O_2 available at the beginning is 0,274 261 mg/ml × 160 ml = 4 341,847 6 mg (1,373 05 mM). The amount dissolved in the seawater can be neglected. This amount of O_2 is sufficient to oxidize to CO_2 and H_2O an amount of biodegradable organic carbon equal to 16,441 566 mg and producing 1,373 05 × 44 = 60,285 742 mM mg of CO_2.

The system needs to be opened in order to refresh the headspace when the O_2 concentration reaches 25 % of the original oxygen concentration.



Key

1 sediment
2 sample
3 cover slip
4 liquid medium
5 container for the CO_2 absorber

Figure A.1 — Test flask

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