
**Plastics — Determination of the
aerobic biodegradation of plastic
materials exposed to seawater —**

**Part 1:
Method by analysis of evolved carbon
dioxide**

*Plastiques — Détermination de la biodégradation aérobie des
matières plastiques exposées à l'eau de mer —*

Partie 1: Méthode par analyse du dioxyde de carbone dégagé



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of 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 www.iso.org/iso/foreword.html.

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

A list of all parts in the ISO 23997 series can be found on the ISO website.

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

According to the United Nations Environment Program (UNEP), one of the most notable properties of synthetic polymers and plastics is their durability which, combined with their accidental loss, deliberate release and poor waste management has resulted in the ubiquitous presence of plastic in oceans (UNEP, 2015^[16]).

It is well known and documented that marine litter can pose risks and a negative impact on living marine organisms and on human beings. Degradability of plastic materials exposed to the marine environment is one of the factors affecting impact and strength of effects. 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 are of interest in order to better characterize the behaviour of plastics in these very particular environments. Thus, the degree and rate of biodegradation is of major interest in order to obtain an indication of the potential biodegradability of plastic materials when exposed to different marine habitats.

ISO/TC 61/SC 14 has established several test methods for biodegradation testing of plastic materials under laboratory conditions covering different environmental compartments and test conditions, as shown in [Table 1](#).

Table 1 — Test methods for biodegradation testing of plastics

Conditions		Test methods
Environmental compartment	Presence/absence of oxygen	
Controlled composting conditions	Aerobic conditions	ISO 14855-1
		ISO 14855-2
High-solids anaerobic-digestion conditions	Anaerobic conditions	ISO 15985
Controlled anaerobic slurry system	Anaerobic conditions	ISO 13975
Soil	Aerobic conditions	ISO 17556
Aqueous medium	Aerobic conditions	ISO 14851
		ISO 14852
	Anaerobic conditions	ISO 14853
Seawater/sandy sediment interface	Aerobic conditions	ISO 18830 ^a
		ISO 19679 ^a
Marine sediment	Aerobic conditions	ISO 22404 ^a
Seawater	Aerobic conditions	ISO 23977-1 ^a
		ISO 23977-2 ^a
^a Test method for measuring biodegradation of plastic materials when exposed to marine microbes.		

All marine biodegradation test methods are based on exposure of plastic materials to marine samples (seawater and/or sediment) taken from shoreline areas. By a quantitative viewpoint, these methods are not equivalent, because, for example, the microbial density in seawater is generally lower compared to the density determined in sediment. In addition, the microbial composition and diversity can be different. Moreover, as a rule, the nutrient concentration found in sediment is normally higher compared to the concentration in seawater.

This document provides a test method for determining the biodegradation level of plastic materials exposed to the microbial population present in seawater from a pelagic zone under laboratory conditions. The biodegradation is followed by measuring the evolved CO₂.

The test is performed with either seawater only (“pelagic seawater test”) or with seawater to which little sediment was added (“suspended sediment seawater test”).

The pelagic seawater test simulates the conditions found in offshore areas with low water currents and low tidal movements, whereas the suspended sediment seawater test simulates conditions which might be found in coastal areas with stronger water currents and tidal movements.

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Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater —

Part 1: Method by analysis of evolved carbon dioxide

1 Scope

This document specifies a laboratory test method for determining the degree and rate of the aerobic biodegradation level of plastic materials. Biodegradation is determined by measuring the CO₂ evolved from plastic materials when exposed to seawater sampled from coastal areas under laboratory conditions.

The conditions described in this document might not always correspond to the optimum conditions for the maximum degree of biodegradation, however this test method is designed to give an indication of the potential biodegradability of plastic materials.

NOTE This document addresses plastic materials but can also be used for other materials.

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 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

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*

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

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]

3.2
dissolved inorganic carbon
DIC

part of the inorganic 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·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:—, 3.4]

3.3
theoretical amount of evolved carbon dioxide
ThCO₂

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

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

[SOURCE: ISO 14852:—, 3.5]

3.4
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.

[SOURCE: ISO 17556:2019, 3.14]

3.5
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·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:—, 3.7]

3.6
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* (3.8)

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:—, 3.8]

3.7
biodegradation phase

time from the end of the *lag phase* (3.6) of a test until the plateau phase has been reached

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:—, 3.10]

3.8**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:—, 3.9]

3.9**plateau phase**

time from the end of the *biodegradation phase* (3.7) until the end of a test

Note 1 to entry: It is measured in days.

[SOURCE: ISO 14852:—, 3.11]

3.10**pre-conditioning**

pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatization of the microorganisms to the test conditions

[SOURCE: ISO 14852:—, 3.13]

4 Principle

This document describes two variations of a test method for determining the biodegradability of plastic materials by the indigenous population of microorganisms in natural seawater using a static aqueous test system. The test is performed under mesophilic test conditions for up to two years by incubating plastic materials with either seawater only ("pelagic seawater test") or with seawater to which low amount of sediment has been added ("suspended sediment seawater test"), coming from the same site as that from which the seawater was taken.

Biodegradation is followed by measuring the evolution of carbon dioxide using a suitable, analytical method. The level of biodegradation is determined by comparing the amount of carbon dioxide evolved with the theoretical amount [theoretical amount of evolved carbon dioxide (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:—, Annex A.

5 Test environment

Incubation shall take place in the dark or in diffused light, in an enclosure which is free from vapours inhibitory to marine microorganisms and which is maintained at a constant mesophilic temperature. It should preferably be between 15 °C to 25 °C, but not exceeding 28 °C, to an accuracy of ± 1 °C. Any change in temperature shall be justified and clearly indicated in the test report.

NOTE Test results are obtained for temperatures that can be different from real conditions in marine environment.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Water

Distilled or deionized water, free of toxic substances (copper in particular) and containing less than 2 mg/l of TOC.

6.2 Natural seawater/sediment

Sampling, preservation, handling, transport and storage of natural seawater, and, if applicable, sediment collected from the same site as that from which the seawater is taken, shall be in accordance with ISO 5667-3.

Prior to use, remove coarse particles from the seawater and, if applicable, from the sediment by appropriate means. The procedure used shall be reported.

Seawater can be filtered using a paper filter in order to remove coarse particles. It is recommended to reduce the amount of coarse particles in sediment by means of at least two washing steps using filtered seawater without coarse particles.

Measure TOC, pH and nitrogen content of seawater and, if applicable, of sediment samples according to ISO 8245, ISO 10523 and ISO 11261, respectively.

If the TOC content of the seawater sample is found to be high, the seawater should be pre-conditioned for about a week prior to use. If, for instance, the background concentration of TOC exceeds about 20 % of the total TOC after addition of the test item, then pre-condition the seawater and, if applicable, the sediment by stirring under aerobic conditions at the test temperature and in the dark or in diffuse light in order to reduce the content of easily degradable organic material.

Provide the following information on the seawater, and, if applicable, on the sediment sample itself:

- date of collection;
- depth of collection (m);
- appearance of sample - turbid, clear, etc.;
- temperature at the time of collection (°C);
- salinity (PSU);
- total organic carbon (TOC; mg/l);
- nitrogen (total-N; mg/l);
- pH;
- description of the pre-conditioning process, if applicable.

7 Apparatus

Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter. Required is usual laboratory equipment, plus the following.

7.1 Test flasks. Biometric flasks of the volume of about 300 ml are appropriate. The vessels shall be located in a constant temperature room or in an apparatus fitted with a thermostat (e.g. water-bath).

Reactors with higher or lower volumes can be used, if environmental conditions are not affected.

7.2 Container for the CO₂ absorber, (e.g. glass beaker) to be located in the headspace of a test flask and filled with 10 ml of Ba(OH)₂ 0,012 5 mol/l or 3 ml of KOH 0,5 mol/l.

As an alternative to Ba(OH)₂ and KOH 4 ml of NaOH 1 mol/l can be used as a CO₂-absorber.

A suitable apparatus is shown in [Annex A, Figure A.1](#).

7.3 Analytical equipment for determining carbon dioxide, consisting of any suitable apparatus with sufficient accuracy, such as a CO₂ or dissolved inorganic carbon (DIC) analyser or apparatus for titrimetric determination after complete absorption in a basic solution, shall be used.

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

7.5 Magnetic stirrer.

7.6 pH meter.

8 Procedure

8.1 Test material

The sample shall be of known mass and contain enough carbon to yield CO₂ that can be adequately measured by the chosen system. Use a test material concentration of at least 100 mg per litre of seawater. 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 in the glass flask. The recommended amount per litre seawater is 150 mg to 300 mg of test material per litre seawater. Calculate the TOC from the chemical formula or determine it by means of a suitable analytical technique (e.g. elemental analysis or measurement in accordance with ISO 8245) and calculate the ThCO₂.

The test material is added to a test flask, either as powder or in the form of a film. If the test material is used in the form of powder, particles of known, narrow size distribution should be used. A particle-size distribution with a maximum diameter of 250 µm is recommended. The preparation of powder shall be performed in accordance with ISO 10210. If the test material is used in the form of a film, it can be added either as pieces in the range of 0,2 cm × 0,2 cm to 0,5 cm × 0,5 cm or as a single plastic strip (width: approximately 1,0 cm, length: depending on weight of the polymer and thickness of the film). It is recommended that the plastic strip is fixed in, for example, a Polytetrafluoroethylene (PTFE) coated fibre net¹⁾ (size: approximately 4 cm × 9 cm, mesh size: 5 mm × 5 mm). The fibre net is folded into 2 layers (approximately 2 cm × 9 cm) with the plastic strip test material fixed in between. Then, the two ends of the fibre net are attached together. The test material fixed between the fibre net is placed upright on the ground of a bottle base in the form of a cylinder (see [Annex A, Figure A.2](#)).

The form and shape of the test material can influence its biodegradability. Similar particle sizes of power should preferably be used in the test. Similar shapes and thicknesses of the films should preferably be used if different kinds of plastic materials are to be compared.

When powder or pieces of films are used in the test, particles or film pieces can stick on the inner wall of the testing bottle above the seawater. In such cases, a slight manual shaking of the bottle is recommended to regain the powder or film pieces back to the seawater sample. If the material is added as a cylindrical plastic strip fixed between, such as a Polytetrafluoroethylene (PTFE) coated fibre net (see [Annex A, Figure A.2](#)), it is immersed in the seawater most of the time.

8.2 Reference materials

Use microcrystalline cellulose or ashless cellulose filters as a reference material²⁾. If possible, the TOC, form, and size should be comparable to that of the test material.

1) PTFE Glass Fabric (product no 9002) produced by Fiberflon (<https://www.fiberflon.de/Products/PTFE-Coated-Open-Mesh-Fabrics/Page-307-17.aspx>) 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.

2) Microcrystalline Cellulose "Avicel" produced by Merck or laboratory filter paper Whatman n° 42 has been found satisfactory for this purpose and are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

As a negative control, a non-biodegradable polymer (e.g. polyethylene) in the same form as the test material can be used.

8.3 Test set up

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, if biodegradation is expected to take longer than 6 months, it is recommended that a negative control is included:

- 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.

8.4 Pre-conditioning phase

As a rule, use a test flask with a volume of 300 ml.

The test is performed in batch by incubating the test materials with either 90 ml of natural seawater only ("pelagic seawater test") or with 90 ml of natural seawater to which sediment of 0,1 g/l to 1,0 g/l (wet weight) has added ("suspended sediment seawater test").

Add carbon dioxide absorber to the absorber compartments of the test flask, as a rule 10 ml $\text{Ba}(\text{OH})_2$ 0,012 5 mol/l, 3 ml of KOH 0,5 mol/l or 4 ml of NaOH 1,0 mol/l. Place the sealed flasks on a magnetic stirrer (7.5) in a constant-temperature environment and allow all vessels to reach the desired temperature. Agitation shall be continuous (e.g. 100 r/min agitation) in order to maintain microorganisms and, if applicable, sediment in suspension.

The abrasion of sediment in coastal areas is a natural phenomenon caused by water currents and tidal movements. Nevertheless, if a magnetic stirring bar is used to mix the seawater to which sediment has added ("suspended sediment seawater test"), it is recommended that either a PTFE-coated dumbbell shaped magnetic stirring bar be used or a PTFE-coated magnetic bar equipped with a pivot ring in order to reduce excessive abrasion of sediment during the test period. Other stirring systems can be used, too. Examples of suitable set-ups are given in Briassoulis D. et.al.^[17] and OECD TG 308:2002, Annex 4^[14].

Take the necessary readings and monitor the CO_2 evolution. This phase is carried out to verify that the endogenous respiration is similar in the different vessels. In addition, the background concentration of easily degradable organic material in natural seawater and, if applicable, in sediment is reduced in this phase, following the pre-conditioning procedure given in 6.2.

8.5 Start of the test

After the pre-conditioning phase open the flasks and add the test material either as powder or in the form of a film to the test flasks (7.1). The mass of samples shall be about 20 mg test material when using a flask with a volume of 300 ml corresponding to an initial test item concentration specified in 8.1.

Repeat the procedure for the reference material and, if applicable, for the material of the negative control. Record the mass of the test sample, the volume of seawater and, if applicable, the mass of the sediment which has been added to each flask.

It is recommended to add KH_2PO_4 (0,1 g/l) and NH_4Cl (0,05 g/l) to seawater samples at the beginning of a test.

Measure the pH of the seawater using ISO 10523, and, if necessary, adjust the pH to a range of between pH 6,0 and pH 8,0 using hydrogen chloride (HCl) or sodium hydroxide (NaOH) solutions.

During the test period, nutrients may be supplemented as needed to support microbial diversity and to maintain the capacity of biodegrading the test material. Take care that the ratio of carbon in the test, reference and, if applicable, the negative control material to nitrogen in the medium is at least about C:N = 40:1. Add nitrogen such as NH_4Cl or NaNO_3 if required.

In addition, if a long lag phase is expected before a significant biodegradation of the test material can be measured, part of the seawater (e.g. about 20 %) and, if applicable, of the sediment (e.g. about 20 %) may be periodically replaced with fresh seawater and sediment, in order to reduce possible depletion of essential nutrients and to maintain the diversity of the microbial community. If seawater and, if applicable, sediment is replaced it shall be replaced in all test material, reference material and blank flasks. Take appropriate measures to ensure that the test material remains in the test flasks (7.1) during the exchange of seawater. The replacement of seawater in the test vessel can be performed by means of a careful removal of seawater by using a pipette and visual inspection that test material is not being removed. Sediment can be replaced by using forceps. It is recommended that the replacement of seawater and sediment is stopped when the lag phase is finished, and the biodegradation phase has started.

The need for and the timing of additional nutrients or other appropriate measures may be judged by observing the temporal course of the biodegradation curve of the reference material.

Any addition of nutrients and applied measures shall be reported in the test report.

8.6 Carbon dioxide measurement

8.6.1 The CO_2 reacts with $\text{Ba}(\text{OH})_2$ 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 or NaOH instead of $\text{Ba}(\text{OH})_2$, which do not form a precipitate.

It is recommended that the evolved CO_2 is determined by means of an automatic titrator in order to avoid occupational exposure with phenolphthalein. The substance is classified as carcinogenic (category 1B) according to the UN Globally Harmonized System for Classification and Labelling of Chemicals (GHS)^[15].

8.6.2 The containers for the CO_2 (7.2) absorber shall be removed and titrated before their capacity is exceeded. The period of time will vary with seawater and test materials and increases slowly as the carbon content of the seawater is reduced. At the time of removal of the containers, the reactor should be allowed to remain 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 End of the test

When a constant level of CO_2 evolution is observed (plateau phase reached) and no further biodegradation is expected, the test is considered complete. The test period should typically not exceed 1 year. However, if significant biodegradation is still observed and the plateau phase has not been reached after this length of time, then the test may be extended, but not to longer than 2 years. 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 (see 8.5) or to provide sufficient nutrients (see 8.5), shall be detailed in the test report. On the last day of the test, measure the pH of the seawater in all flasks, subsequently acidify the seawater in all flasks 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 following the procedure given in 8.6.

9 Calculation and expression of results

9.1 Calculation

9.1.1 Amount of CO₂ produced

9.1.1.1 Net CO₂ produced

The first step in calculating the amount of CO₂ produced is to correct the test material reactors for endogenous CO₂ production. The control reactor serves as a blank to correct for CO₂ which may be produced through endogenous respiration of the microorganisms. The amount of CO₂ 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 of HCl titrated into mg of CO₂ produced.

9.1.1.2 Ba(OH)₂ used as CO₂ absorber

When CO₂ enters the absorber containers, it reacts in the following manner, see [Formula \(1\)](#):



where s = solid.

The BaCO₃ s formed is insoluble and precipitates.

The amount of Ba(OH)₂ remaining in the solution is determined by titration of the 10 ml of the CO₂ absorber with HCl according to the following chemical reaction, see [Formula \(2\)](#):



The amount of the remaining Ba(OH)₂ is determined with the [Formula \(3\)](#):

$$R_t = \frac{\text{mol HCl}}{2} \quad (3)$$

where R_t is the remaining Ba(OH)₂.

The amount of the reacted Ba(OH)₂ is determined as the difference between the amount originally present in the absorber and the amount remaining after reaction with CO₂, see [Formula \(4\)](#).

$$R_r = R_0 - R_t \quad (4)$$

where

R_r is the amount of reacted Ba(OH)₂;

R_0 is the amount of Ba(OH)₂ originally present in the absorber;

R_t is the amount of Ba(OH)₂ remaining after the reaction with CO₂.

This means that the number of mol of CO₂ produced is derived using [Formula \(5\)](#):

$$\text{mol CO}_2 = R_r \quad (5)$$

where R_r is the amount of reacted Ba(OH)₂.

9.1.1.3 KOH used as CO₂ absorber

The evolved CO₂ will react with KOH in the following manner, see [Formula \(6\)](#):



K₂CO₃, the product of [Formula \(6\)](#), is soluble and does not precipitate.

The fresh KOH solution, where no CO₂ has been absorbed, can be titrated with HCl as, see [Formula \(7\)](#):



The KOH solutions used as CO₂ absorbers will have both unreacted KOH and K₂CO₃ as per [Formula \(6\)](#).

During titration, both chemical species will react with HCl, as follows, see [Formula \(8\)](#) and [Formula \(9\)](#):



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 CO₂ 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 [\(9\)](#), as shown in [Formula \(10\)](#). In practice:

$$\text{mmol CO}_2 = (V_{\text{HCl}(7)} - V_{\text{HCl}(8+9)}) \times [\text{HCl}] \quad (10)$$

where

$V_{\text{HCl}(7)}$ is the volume of HCl (expressed in ml) consumed in [Formula \(7\)](#);

$V_{\text{HCl}(8+9)}$ is the volume of HCl (expressed in ml) consumed in [Formulae \(8\)](#) and [\(9\)](#);

$[\text{HCl}]$ is the concentration of the HCl solution (0,05 mol/l).

If an end point titrator is available, the mmol of CO₂ can be determined, without using an indicator, with a further reaction. A further addition of HCl makes HCl react with KHCO₃, produced with [Formula \(9\)](#), see [Formula \(11\)](#):



The number of equivalent consumed in [Formula \(11\)](#), and therefore in [Formula \(9\)](#), corresponds to the K₂CO₃ produced during [Formula \(6\)](#), that in turn corresponds to the absorbed CO₂.

Consequently, 1 mol of KHCO₃ corresponds to 1 mol of CO₂ reacted in [Formula \(6\)](#), and thus the mmol CO₂ are equivalent to the mmol HCl consumed in [Formula \(11\)](#) end point.

Therefore, the amount of CO₂ can be calculated using [Formula \(12\)](#):

$$mmol\ CO_2 = (V_{HCl(11)}) \times [HCl] \quad (12)$$

where

$V_{HCl(11)}$ is the volume of HCl (expressed in ml) consumed in [Formula \(11\)](#);

$[HCl]$ is the concentration of the HCl solution (0,05 mol/l).

The amount of CO₂ expressed in mg is finally obtained using [Formula \(13\)](#):

$$mgCO_2 = mmol\ CO_2 \times 44 \quad (13)$$

where 44 is the molecular weight (g/mol) of CO₂.

When NaOH is used as a CO₂ absorber, [Formula \(6\)](#) to [Formula \(13\)](#) apply, too, if the symbol for potassium (K) is replaced by the symbol for sodium (Na).

9.1.2 Percentage of biodegradation

The percentage of biodegradation is the ration between the evolved CO₂ and theoretical CO₂ (ThCO₂).

The ThCO₂ is shown in [Formula \(14\)](#) and percentage of biodegradation in [Formula \(15\)](#):

$$ThCO_2 = S \times TOC(\%) \times \frac{44}{12} \quad (14)$$

where

S is the amount of specimen (mg);

$TOC(\%)$ is the TOC of the plastic material (or reference material or, if applicable, negative control material) divided by 100;

44 is the molecular weight of CO₂;

12 is the molecular weight of C.

Therefore, see [Formula \(15\)](#):

$$\%B = \frac{CO_2}{ThCO_2} \times 100 \quad (15)$$

where

$\%B$ is the percentage of biodegradation;

CO_2 is the CO_2 produced, expressed in mg;

$ThCO_2$ is the theoretical amount of evolved CO_2 .

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, for example 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:

- the degree of biodegradation of the reference material (F_C) is $> 60 \%$ after 180 days;
- the evolved CO_2 of the blank F_B at the end of the test does not exceed $150 \text{ mg } CO_2/\text{l}$ seawater after 6 months;
- the maximum difference of the amount of CO_2 evolved in replicate vessels of the blank (F_B) at the plateau phase or at the end of the test is less than 20% ;
- the maximum difference of biodegradation in replicate vessels of the reference material (F_C) at the plateau phase or at the end of the test is less than 20% ;
- the degree of biodegradation of the negative control (flasks F_N) is below 10% at the end of the test.

If these criteria are not fulfilled, repeat the test using a different kind of natural seawater.

11 Test report

The test report shall contain at least the following information:

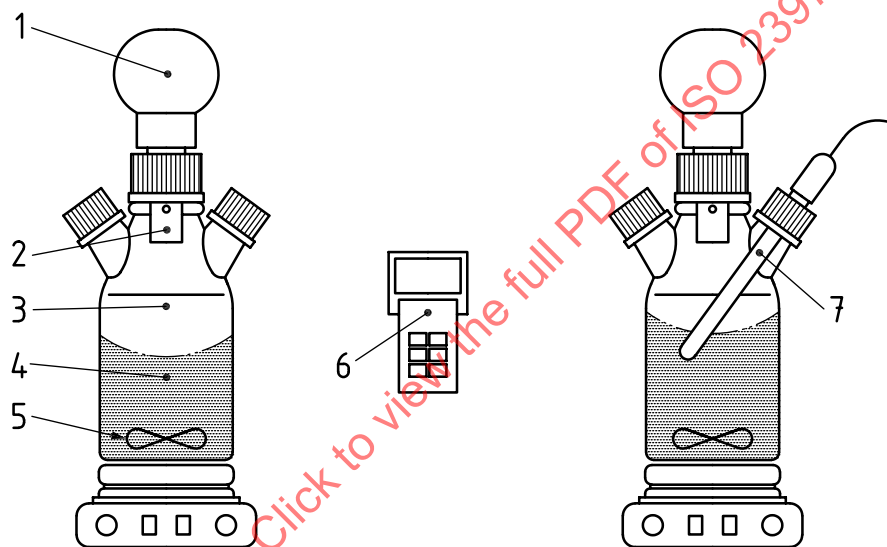
- a) a reference to this document, i.e. ISO 23977-1:2020;
- b) all information necessary to identify the test and reference materials, including their TOC, ThCO_2 , chemical composition and formula (if known), shape, form and amount in the samples tested;
- c) the source of seawater and, if applicable, marine sediment (see 6.2);
- d) description of the pre-conditioning phase, if applicable (see 8.4);
- e) whether the test has been performed as a pelagic seawater test (without addition of sediment) or as suspended sediment seawater test (with addition of sediment);
- f) the amount of sediment added to seawater, if applicable;
- g) the main test parameters, including test volume, incubation temperature and final pH;
- h) the analytical techniques used, including the principle of the respirometer and the TOC;
- i) 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;
- j) 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 ;
- k) 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);
- l) details of the methods used during the test period in order to support microbial diversity or to avoid nutrient deficiency (see 8.5);
- m) any deviations from the test conditions described in this document.

Annex A (informative)

Example of a respirometric system

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 seawater, and if applicable, in the sediment consume the oxygen and form CO_2 . This is absorbed by a CO_2 absorber [generally $\text{Ba}(\text{OH})_2$, KOH or NaOH] that can then be titrated to determine the amount of the absorbed CO_2 .

In a typical case, a 300 ml vessel is used filled with about 90 ml of seawater from a pelagic zone and, if applicable, with sediment (wet, 0,1 g/l to 1,0 g/l). The headspace is about 210 ml.



Key

- 1 pressure measuring head with IR interface
- 2 sorption container for CO_2 -sorption
- 3 headspace
- 4 seawater
- 5 magnetic stirrer
- 6 controller with IR interface
- 7 pH electrode (optional version with pH measurement)

Figure A.1 — Example of a respirometry apparatus