INTERNATIONAL STANDARD

ISO 23977-2

First edition
2020-11

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

Part 2:
Method by measuring the oxygen demand in closed respirometer

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

Partie 2: Méthode par mesure de la demande en oxygène dans un respiromètre fermé

Reference number
ISO 23977-2:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

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[15]).

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 biodegradables 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 oxygen demand in a closed respirometer.

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.

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

Part 2:
Method by measuring the oxygen demand in closed respirometer

1 Scope

This document specifies a laboratory test method for determining the degree and rate of the aerobic biodegradation level of plastic materials. Biodegradation of plastic materials is determined by measuring the oxygen demand in a closed respirometer 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
biochemical oxygen demand
BOD
mass concentration of the dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in water

Note 1 to entry: It is expressed as milligrams of oxygen uptake per milligram or gram of test compound.

[SOURCE: ISO 18830:2016, 3.1]

3.3
theoretical oxygen demand
ThOD
theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula

Note 1 to entry: It is expressed as milligrams of oxygen uptake per milligram or gram of test compound.

[SOURCE: ISO 18830:2016, 3.2]

3.4
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 $m \cdot s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.

[SOURCE: ISO 14852:—, 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.

The system is contained in a closed flask, in a respirometer. The carbon dioxide evolved is absorbed in a suitable absorber in the headspace of the flasks. The consumption of oxygen (BOD) is determined, for example, by measuring the amount of oxygen required to maintain a constant volume of gas in the respirometer flasks, or by measuring the change in volume or pressure (or a combination of the two) either automatically or manually.

The level of biodegradation is determined by comparing the BOD with the theoretical amount (ThOD) and expressed in percentage. The influence of possible nitrification processes on the BOD shall be considered. The test result is the maximum level of biodegradation determined from the plateau phase of the biodegradation curve.

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 Closed respirometer.

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.

A suitable apparatus is shown in Annex A, Figure A.1.

Any respirometer able to determine with sufficient accuracy the biochemical oxygen demand is suitable, preferably an apparatus which measures and replaces automatically and continuously the oxygen consumed so that no oxygen deficiency and no inhibition of the microbial activity occurs during the degradation process. Analytical equipment to measure total organic carbon (TOC) and dissolved organic carbon (DOC) is given in ISO 8245.

7.2 Analytical equipment for measuring nitrate and nitrite concentrations.

A qualitative test (for example test strips or photometric tests for nitrate and nitrite) is recommended first to decide if any nitrification has occurred. If there is evidence of nitrate/nitrite in the medium, a quantitative determination using a suitable method (for example ion chromatography) is required.

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

7.4 Magnetic stirrer.

7.5 pH meter.

8 Procedure

8.1 Test material

The sample shall be of known mass and contain sufficient carbon to yield a BOD that can be adequately measured by the chosen system [closed respirometer (7.1)].

Use a test material concentration of at least 100 mg/l of seawater plus sediment. The mass of the samples should correspond to a ThOD of about 170 mg/l or a TOC of about 60 mg/l. The maximum mass of sample per flask is limited by the oxygen supply to the respirometer.

The ThOD (see ISO 14851:2019, Annex A) and the TOC (using ISO 8245 or from the chemical formula or determine by elemental analysis) shall be calculated.

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 net[1] (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, for example, a polytetrafluoroethylene (PTFE) coated fibre net (see Annex A, Figure A.2), it is immersed in the seawater most of the time.

---

[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.

8.2 Reference materials

Use microcrystalline cellulose or ashless cellulose filters as a reference material[2]. 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 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 (see ISO 14851:2019, Annex C). Place the sealed flasks on a magnetic stirrer (7.4) 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[16]. and OECD TG 308:2002, Annex 4[14].

Take the necessary readings on the manometers (if manual) and verify that the recorder of oxygen consumption is functioning properly (automatic respirometer). 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.

---

[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.

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. 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 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 End of the test

When a constant level of BOD 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. At the end of the test, measure the pH and determine the concentrations of nitrate and nitrite immediately in the seawater of all flasks, or take suitably preserved samples. Use the values to correct the calculated degree of biodegradation for nitrification (see ISO 14851:2019, Annex B).

9 Calculation and expression of results

9.1 Calculation

Read the oxygen consumption values for each flask, using the method given by the manufacturer for the appropriate type of respirometer. Calculate the specific biochemical oxygen demand (BOD_S) of the test material as the difference between oxygen consumption in the test flasks F_T and the blanks F_B divided by the concentration of the test material, using Formula (1):

$$\mathrm{BOD_S} = \frac{\mathrm{BOD_t} - \mathrm{BOD_{Bt}}}{\rho_{\mathrm{TC}}} \tag{1}$$

where

$\mathrm{BOD_S}$	is the specific BOD, in mg per milligram of test material;
$\mathrm{BOD_t}$	is the BOD of the flasks F_T containing test material at time *t*, in mg/l;
$\mathrm{BOD_{Bt}}$	is the BOD of the blank F_B at time *t*, in mg/l;
ρ_{TC}	is the concentration of the test material in the reaction mixture of flask F_T, in mg/l.

Calculate the percentage biodegradation, D_t, as the ratio of the specific biochemical oxygen demand to the theoretical oxygen demand (ThOD, in mg per gram of test material), using Formula (2):

$$D_{\mathrm{t}} = \frac{\mathrm{BOD_S}}{\mathrm{ThOD}} \times 100 \tag{2}$$

Calculate, in the same way, the BOD and percentage biodegradation of the reference material F_C and, if included, the negative control F_N.

NOTE For calculation of the ThOD, see ISO 14851:2019, Annex A. If significant concentrations of nitrite and nitrate are determined, consider the oxygen demand due to nitrification (see ISO 14851:2019, Annex B).

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 BOD values measured and the percentages of biodegradation for each measurement interval and each test flask. For each vessel, plot a BOD 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, such as 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 BOD of the blank F_B at the end of the test does not exceed an upper limiting value;

NOTE This value depends on the amount of inoculum; in typical suspended sediment seawater tests supplemented with 1 g/l sediment (wet weight), the BOD of the blank FB was about 40-80 mg/l after 6 months.

c) the maximum difference of the amount of BOD in replicate vessels of the blank (F_B) at the plateau phase or at the end of the test is less than 20 %;

d) 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 %;

e) 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 23977-2:2020;

b) all information necessary to identify the test and reference materials, including their TOC, ThOD, 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 and nitrate/nitrite determinations;

i) all the test results obtained for the test and reference materials (in tabular and graphical form), including the measured BOD, the percentage biodegradation values, the respective curves of these parameters against time and the nitrate/nitrite concentrations;

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);

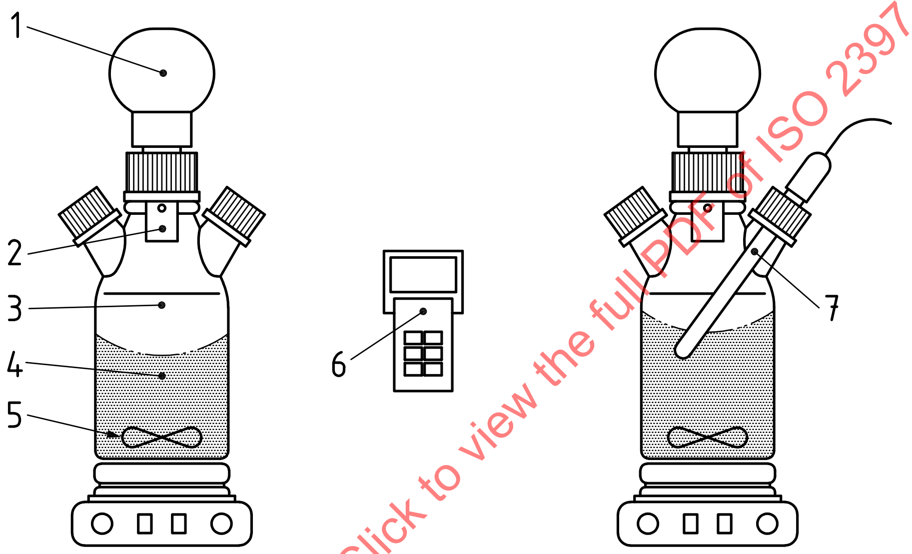
m) any deviations from the test conditions described in this document.

Annex A
(informative)

Example of a respirometric system

Biological oxygen demand (BOD) respirometers can be based on a pressure measurement in a closed system. Microorganisms in the vessel consume O_2 and form CO_2. This is absorbed by a CO_2 absorber (generally NaOH), creating a vacuum which can be read directly as a measured value in mg/l BOD.

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