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Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer

Évaluation de la biodégradabilité aérobie ultime des matériaux plastiques en milieu aqueux — Méthode par détermination de la demande en oxygène dans un respiromètre fermé

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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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 14851 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 5, *Physical-chemical properties*.

Annexes A to G of this International Standard are for information only.

This corrected version of ISO 14851:1999 incorporates the following corrections:

— in Clause 2, the year of publication of ISO 9408 has been inserted and the footnote deleted;

— the remaining footnotes have been renumbered;

— in Annex C, errors in the key to Figure C.1 have been corrected and minor improvements made to the figure itself;

— in the Bibliography, references [1] and [2] have been updated.

Introduction

With the increasing use of plastics, their recovery and disposal have become a major issue. As a first priority, recovery should be promoted. Complete recovery of plastics, however, is difficult. For example, plastic litter, which comes mainly from consumers, is difficult to recover completely. Additional examples of plastics which are difficult to recover are fishing tackle, agricultural mulches and water-soluble polymers. These plastic materials tend to leak from closed waste-management cycles into the environment. Biodegradable plastics are now emerging as one of the options available to solve such environmental problems. Plastic materials, such as products or packaging, which are sent to composting facilities should be potentially biodegradable. Therefore it is very important to determine the potential biodegradability of such materials and to obtain an indication of their biodegradability in natural environments.

Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer

WARNING — Sewage, activated sludge, soil and compost may contain potentially pathogenic organisms. Therefore appropriate precautions should be taken when handling them. Toxic test compounds and those whose properties are unknown should be handled with care.

1 Scope

This International Standard specifies a method, by measuring the oxygen demand in a closed respirometer, for the determination of the degree of aerobic biodegradability of plastic materials, including those containing formulation additives. The test material is exposed in an aqueous medium under laboratory conditions to an inoculum from activated sludge, compost or soil.

If an unadapted activated sludge is used as the inoculum, the test simulates the biodegradation processes which occur in a natural aqueous environment; if a mixed or pre-exposed inoculum is used, the method can be used to investigate the potential biodegradability of a test material.

The conditions used in this International Standard do not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but the standard is designed to determine the potential biodegradability of plastic materials or give an indication of their biodegradability in natural environments.

The method enables the assessment of the biodegradability to be improved by calculating a carbon balance (optional, see annex E).

The method applies to the following materials:

— Natural and/or synthetic polymers, copolymers or mixtures thereof.

— Plastic materials which contain additives such as plasticizers, colorants or other compounds.

— Water-soluble polymers.

— Materials which, under the test conditions, do not inhibit the microorganisms present in the inoculum. Inhibitory effects can be determined using an inhibition control or by another appropriate method (see e.g. ISO 8192[3]). If the test material is inhibitory to the inoculum, a lower test concentration, another inoculum or a pre-exposed inoculum can be used.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 8245:1999, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).*

ISO 9408:1999, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer.*

ISO 10634:1995, *Water quality — Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium.*

ISO/TR 15462:1997, *Water quality — Selection of tests for biodegradability.*

3 Definitions

For the purposes of this International Standard, the following definitions apply:

3.1
ultimate aerobic biodegradation
the breakdown of an organic compound by microorganisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new biomass

3.2
activated sludge
biomass produced in the aerobic treatment of waste water by the growth of bacteria and other microorganisms in the presence of dissolved oxygen

3.3
concentration of suspended solids in an activated sludge
the amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105 °C to constant mass

3.4
biochemical oxygen demand
BOD
the mass concentration of the dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in water, expressed as milligrams of oxygen uptake per milligram or gram of test compound

3.5
theoretical oxygen demand
ThOD
the theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated from the molecular formula, expressed as milligrams of oxygen uptake per milligram or gram of test compound

3.6
total organic carbon
TOC
all the carbon present in organic matter which is dissolved or suspended in water

3.7
dissolved organic carbon
DOC
that part of the organic carbon in water which cannot be removed by specified phase separation, 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

3.8
lag phase
the time, measured in days, 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

3.9
maximum level of biodegradation
the degree of biodegradation, measured in per cent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test

3.10
biodegradation phase
the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached

3.11
plateau phase
the time, measured in days, from the end of the biodegradation phase until the end of a test

3.12
pre-exposure
the pre-incubation of an inoculum in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the microorganisms

3.13
pre-conditioning
the 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

4 Principle

The biodegradability of a plastic material is determined using aerobic microorganisms in an aqueous system. The test mixture contains an inorganic medium, the organic test material (the sole source of carbon and energy) with a concentration between 100 mg/l and 2 000 mg/l of organic carbon, and activated sludge or a suspension of active soil or compost as the inoculum. The mixture is stirred in closed flasks in a respirometer for a period not exceeding 6 months. 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. An example of a respirometer is given in annex C. Alternatively, the two-phase closed-bottle version described in ISO 10708[4] may be used (see annex D).

The level of biodegradation is determined by comparing the BOD with the theoretical amount (ThOD) and expressed in per cent. The influence of possible nitrification processes on the BOD have to be considered. The test result is the maximum level of biodegradation determined from the plateau phase of the biodegradation curve. Optionally, a carbon balance may be calculated to give additional information on the biodegradation (see annex E).

Unlike ISO 9408, which is used for a variety of organic compounds, this International Standard is specially designed for the determination of the biodegradability of plastic materials. The special requirements necessary affect the choice of the inoculum and the test medium, and there is the possibility of improving the evaluation of the biodegradability by calculating a carbon balance.

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 20 °C and 25 °C, to an accuracy of ± 1 °C, or at any other appropriate temperature depending on the inoculum used and the environment to be assessed.

NOTE With a compost inoculum, higher temperatures may be appropriate.

6 Reagents

Use only reagents of recognized analytical grade.

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

6.2 Test medium.

Depending on the purpose of the test, different test media may be used. For example, if simulating a natural environment use the standard test medium (6.2.1). If a test material is used at higher concentrations, use the optimized test medium (6.2.2) with higher buffering capacity and nutrient concentrations.

6.2.1 Standard test medium

6.2.1.1 Solution A

Dissolve

anhydrous potassium dihydrogen phosphate (KH_2PO_4)	8,5 g
anhydrous dipotassium hydrogen phosphate (K_2HPO_4)	21,75 g
disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	33,4 g
ammonium chloride (NH_4Cl)	0,5 g

in water (6.1) and make up to 1000 ml.

NOTE The correct composition of the solution can be checked by measuring the pH, which should be 7,4.

6.2.1.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1) and make up to 1000 ml.

6.2.1.3 Solution C

Dissolve 36,4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (6.1) and make up to 1000 ml.

6.2.1.4 Solution D

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1) and make up to 1000 ml.

Prepare this solution freshly before use to avoid precipitation, or add a drop of concentrated hydrochloric acid (HCl) or a drop of 0,4 g/l aqueous solution of ethylenediaminetetraacetic acid (EDTA).

6.2.1.5 Preparation

To prepare 1 litre of test medium, add, to about 500 ml of water (6.1),

— 10 ml of solution A;

— 1 ml of each of solutions B to D.

Make up to 1000 ml with water (6.1).

6.2.2 Optimized test medium

This optimized medium is highly buffered and contains more inorganic nutrients. This is necessary to keep the pH constant in the system during the test, even at high concentrations of the test material. The medium contains about 2400 mg/l of phosphorus and 50 mg/l of nitrogen and is therefore suitable for concentrations in the test material of up to 2000 mg/l of organic carbon. If higher test-material concentrations are used, increase the nitrogen content to keep the C:N ratio at about 40:1.

6.2.2.1 Solution A

Dissolve

anhydrous potassium dihydrogen phosphate (KH_2PO_4)	37,5 g
disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$)	87,3 g
ammonium chloride (NH_4Cl)	2,0 g

in water (6.1) and make up to 1000 ml.

6.2.2.2 Solution B

Dissolve 22,5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water (6.1) and make up to 1000 ml.

6.2.2.3 Solution C

Dissolve 36,4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water (6.1) and make up to 1000 ml.

6.2.2.4 Solution D

Dissolve 0,25 g of iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water (6.1) and make up to 1000 ml (see second paragraph of 6.2.1.4).

6.2.2.5 Solution E (trace-element solution, optional)

Dissolve in 10 ml of aqueous HCl solution (25 %, 7,7 mol/l), in the following sequence:

70 mg of $ZnCl_2$, 100 mg of $MnCl_2 \cdot 4H_2O$, 6 mg of H_3BO_3, 190 mg of $CoCl_2 \cdot 6H_2O$, 3 mg of $CuCl_2 \cdot 2H_2O$, 240 mg of $NiCl_2 \cdot 6H_2O$, 36 mg of $Na_2MoO_4 \cdot 2H_2O$, 33 mg of $Na_2WO_4 \cdot 2H_2O$ and 26 mg of $Na_2SeO_3 \cdot 5H_2O$

and make up to 1000 ml with water (6.1).

6.2.2.6 Solution F (vitamin solution, optional)

Dissolve in 100 ml of water (6.1) 0,6 mg of biotine, 2,0 mg of niacinamide, 2,0 mg of *p*-aminobenzoate, 1,0 mg of panthotenic acid, 10,0 mg of pyridoxal hydrochloride, 5,0 mg of cyanocobalamine, 2,0 mg of folic acid, 5,0 mg of riboflavin, 5,0 mg of DL-thioctic acid and 1,0 mg of thiamine dichloride or use a solution of 15 mg of yeast extract in 100 ml of water (6.1). Filter the solution for sterilization using membrane filters (see 7.4).

NOTE Solutions E and F are optional and are not required if a sufficient concentration of the inoculum is used, e.g. activated sludge, soil or compost. It is recommended that 1 ml portions be prepared and kept refrigerated until use.

6.2.2.7 Preparation

To prepare 1 litre of test medium, add, to about 800 ml of water (6.1),

— 100 ml of solution A;

— 1 ml of each of solutions B to D and, optionally, E and F.

Make up to 1000 ml with water (6.1) and measure the pH.

NOTE The correct composition of the test medium can be checked by measuring the pH, which should be 7,0 ± 0,2.

6.3 Pyrophosphate solution.

Dissolve 2,66 g of anhydrous sodium pyrophosphate ($Na_4P_2O_7$) in water (6.1) and make up to 1000 ml.

6.4 Carbon dioxide absorber, preferably soda lime pellets or another suitable absorbant.

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, including test vessels (glass flasks) fitted with stirrers and all other necessary equipment, and located in a constant-temperature room or in a thermostatted apparatus (e.g. water-bath). For an example, see annex C.

NOTE 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. Instead of an ordinary respirometer, the two-phase closed-bottle version may be used (see annex D).

7.2 Analytical equipment for measuring total organic carbon (TOC) and dissolved organic carbon (DOC) (see ISO 8245).

7.3 Analytical equipment for measuring nitrate and nitrite concentrations.

NOTE A qualitative test 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.

7.4 Centrifuge, or **filtration device** with membrane filters (0,45 µm pore size) which neither adsorb nor release organic carbon significantly.

7.5 Analytical balance (usual laboratory equipment).

7.6 pH meter (usual laboratory equipment).

8 Procedure

8.1 Test material

The test material shall be of known mass and contain sufficient carbon to yield a BOD that can be adequately measured by the respirometer used. Calculate from the chemical formula or determine by elemental analysis the ThOD (see annex A) and the TOC (using e.g. ISO 8245). Use a test-material concentration of at least 100 mg/l, corresponding to a ThOD of about 170 mg/l or a TOC of about 60 mg/l. Use lower concentrations only if the sensitivity of the respirometer is adequate. The maximum amount of test material is limited by the oxygen supply to the respirometer and the test medium used. When using the optimized test medium (6.2.2), the test-material

concentration shall be such that the TOC does not exceed about 2000 mg/l, i.e. a C:N ratio of about 40:1. If higher concentrations are to be tested, increase the amount of nitrogen in the test medium.

NOTE 1 If biodegradation processes in natural environments are to be simulated, the use of the standard medium and a test-material concentration of 100 mg/l are recommended.

NOTE 2 The test material should preferably be used in powder form, but it may also be introduced as films, pieces, fragments or shaped articles. The form and shape of the test material may influence its biodegradability. Similar shapes should preferably be used if different kinds of plastic material are to be compared. If the test material is used in the form of a powder, particles of known, narrow size distribution should be used. A particle-size distribution with the maximum at 250 μm diameter is recommended. Also, the size of the test equipment used may depend on the form of the test material. It should be ascertained that no substantial mechanical aberrations occur due to the test conditions, for example due to the type of stirring mechanism used. Processing of the test material (e.g. the use of powder in the case of composites) should not influence significantly the degradation behaviour of the material. Optionally, record the hydrogen, oxygen, nitrogen, phosphorus and sulfur contents and the molecular mass of a polymeric test material, using for example liquid exclusion chromatography (see e.g. ASTM D 3536-91[1] or any other applicable standard method). Preferably, plastic materials without additives such as plasticizers should be tested. When the material does contain such additives, information on their biodegradability will be needed to assess the biodegradability of the polymeric material itself.

For details on how to handle poorly water-soluble compounds, see ISO 10634.

8.2 Reference material

Use aniline and/or a well defined biodegradable polymer (for example microcrystalline cellulose powder, ashless cellulose filters or poly-ß-hydroxybutyrate) as a reference material. 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 inoculum

Activated sludge from a sewage-treatment plant treating predominantly domestic sewage is a suitable source of the inoculum. It is obtained from an active aerobic environment and is available over a wide geographical area in which a broad range of plastic materials has to be tested. Alternatively, soil and/or compost suspensions can be used for inoculation, as with some plastic materials the activity of fungi is important for biodegradation. When biodegradation in a specific waste-treatment system is to be determined, collect the inoculum from that environment.

The inoculum can be prepared from the sources described in 8.3.1 and 8.3.2, or from a mixture of these sources in order to obtain a varied and concentrated microbial flora with sufficient biodegradation activity. If the endogenous respiration of the inoculum is too high, stabilize the inoculum by aeration before use. Harmonize the test temperature with the inoculum used (see note to clause 5).

NOTE It may be useful to determine the colony-forming units (cfu) of the inoculum used. The test mixture should preferably contain about 10^{-6} cfu/ml.

8.3.1 Inoculum from wastewater-treatment plants

Take a sample of activated sludge collected from a well-operated sewage-treatment plant or a laboratory plant handling predominantly domestic sewage. Mix well, keep the sample under aerobic conditions and use preferably on the day of collection (at least within 72 h).

Before use, determine the concentration of suspended solids (use e.g. ISO 11923[5]). If necessary, concentrate the sludge by settling so that the volume of sludge added to the test assay is minimal. Add a suitable volume to obtain suspended solids in the range 30 mg/l to 1000 mg/l in the final mixture.

NOTE 1 When biodegradation processes in a natural environment are to be simulated or when a carbon balance determination (see annex E) is to be carried out, an inoculum concentration of 30 mg/l suspended solids is recommended. As solid matter can interfere with the carbon balance determination, the following procedure for preparing the inoculum is recommended. Take 500 ml of the activated sludge and homogenize for 2 min at medium speed in a blender or in a suitable high-speed mixer. Allow to settle until the supernatant liquid contains no significant amounts of suspended matter, but in any case for at least 30 min. Decant a sufficient volume of the supernatant liquid and add it to the test flasks to obtain a concentration of 1 % (*V*/*V*) to 5 % (*V*/*V*) in the test medium. Avoid carrying over sludge particles.