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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.

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).

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

For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 61, *Plastics*, Subcommittee SC 14, *Environmental aspects*.

This second edition cancels and replaces the first edition (ISO 14851:1999), which has been technically revised. It also incorporates the Technical Corrigendum ISO 14851:1999/Cor.1:2005. The main changes compared to the previous edition are as follows:

— the 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 scope and Clause 8, soil and compost have been excluded for the inoculums used in this document;

— in 8.4, numbers of test flask for the test material and blank control have been changed from two to three;

— references in this document have been updated for latest active version;

— the Bibliography has been updated.

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

With the increasing use of plastics, their recovery and disposal have become a major issue. As a first priority, recovery is 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, plastic microbeads in personal care products 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 are expected to 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 document 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.

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 is used to investigate the potential biodegradability of a test material.

The conditions used in this document do not necessarily correspond to the optimum conditions allowing maximum biodegradation to occur, but this document 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, for example, ISO 8192[2]). 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 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 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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
ultimate aerobic biodegradation
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
amount of solids obtained by filtration or centrifugation of a known volume of *activated sludge* (3.2) and drying at about 105 °C to constant mass

3.4
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.

3.5
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.

3.6
total organic carbon
TOC
amount of carbon bound in an organic compound

3.7
dissolved organic carbon
DOC
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
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)

3.9
maximum level of biodegradation
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
time, measured in days, from the end of the *lag phase* (3.8) of a test until about 90 % of the *maximum level of biodegradation* (3.9) has been reached

3.11
plateau phase
time, measured in days, from the end of the *biodegradation phase* (3.10) until the end of a test

3.12
pre-exposure
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
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 as the inoculum. The mixture is stirred in closed flasks in a respirometer for a period not exceeding 2 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[3] 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 has 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). Moreover, also the absorbed carbon dioxide in the adsorber at the end of the test may be determined to give additional information on the biodegradation (see Annex G).

Unlike ISO 9408[6], which is used for a variety of organic compounds, this document 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.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Distilled or deionized water

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 the following in water (6.1) and make up to 1 000 ml.

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

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 1 000 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 1 000 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 1 000 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 l of test medium, add the following 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 1 000 ml with water (6.1). Prepare the test medium freshly before use. The solutions A up to C may be stored up to 6 months in the dark at room temperature.

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 2 400 mg/l of phosphorus and 50 mg/l of nitrogen and is therefore suitable for concentrations in the test material of up to 2 000 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 the following in water (6.1) and make up to 1 000 ml.

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

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 1 000 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 1 000 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 1 000 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 $Na_2SeO_3 \cdot 5H_2O$.

Make up to 1 000 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).

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

6.2.2.7 Preparation

To prepare 1 l 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 1 000 ml with water (6.1) and measure the pH.

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 1 000 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 thermostated 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 can be used (see Annex D).

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

7.3 Analytical equipment for measuring nitrate and nitrite concentrations.

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, for example, 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 2 000 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.

