

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

ICS 83.080.01

National foreword

This British Standard is the UK implementation of EN ISO 14851:2004. It is identical with ISO 14851:1999, incorporating corrigendum May 2005. It supersedes BS ISO 14851:1999 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee PRI/21, Testing of plastics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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15255	3 September 2004	This corrigendum renumbers BS ISO 14851:1999 as BS EN ISO 14851:2004
	30 June 2008	Implementation of ISO corrigendum May 2005, corrections to subclauses 8.3 and 9.1

English version

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

(ISO 14851:1999)

Evaluation de la biodégradabilité aérobie ultime
des matériaux plastiques en milieu aqueux —
Méthode par détermination de la demande en
oxygène dans un respiromètre fermé
(ISO 14851:1999)

This European Standard was approved by CEN on 21 June 2004

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

The text of ISO 14851:1999 has been prepared by Technical Committee ISO/TC 61, Plastics, of the International Organization for Standardization (ISO) and has been taken over as EN ISO 14851:2004 by Technical Committee CEN/TC 249, Plastics, the Secretariat of which is held by IBN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2005, and conflicting national standards shall be withdrawn at the latest by January 2005.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 14851:1999 has been approved by CEN as EN ISO 14851:2004 without any modifications.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

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:—¹⁾, *Water quality — Evaluation of ultimate aerobic biodegradability of organic compounds in an 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*.

¹⁾ To be published. (Revision of ISO 9408:1991).

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·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 ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$)	33,4 g
ammonium chloride (NH_4Cl)	0,5 g

in water (6.1) and make up to 1 000 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 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 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 ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) 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 ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) 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 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 1 000 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 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.