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Plastics — Evaluation of the action of microorganisms

Plastiques — Évaluation de l'action des micro-organismes

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Foreword

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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 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.

This document was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 6, *Ageing, chemical and environmental resistance*.

This third edition cancels and replaces the second edition (ISO 846:1997), which has been technically revised. The main changes compared to the previous edition are as follows.

— The size of the test specimens has been defined as (50 mm ± 1 mm) × (50 mm ± 1 mm). A fixed size allows the determination of any edge effects associated with the area 5 mm from the outer edge (see new Annex C). In this way, the evaluation of growth on the test specimens is harmonized.

— New Annexes B and C have been added and the old annexes have been renumbered.

— The former Annex C has been updated and renumbered as Annex D.

— Test A only:

Stainless steel coupons acting as negative control specimens have been introduced to provide a reference for where fungal growth occurs in the Petri dish, even though no nutrients have been added to the test design.

The test design does not use an agar-medium any more to provide the source of moisture to allow 95 % ± 5 % relative humidity to be achieved. Instead the test specimens are stored in closed containers that include a water reservoir to provide a relative humidity 95 % ± 5 % around the test specimens during incubation;

A grid has been introduced for use during the evaluation of the area of growth observed on the surface of the test specimens. The use of the grid provides and objective mechanisms for assessing growth and is explained in the new Annex C.

— Test B has been deleted.

— Positive control specimens (test specimens that allow fungal growth) have been introduced to allow the determination of basic fungistatic effects of samples that contain biocides.

— The fungal inoculum has been revised to be consistent with other referenced test standards and changes to the names of fungal strains have been incorporated.

— The media used in the test have been revised based on the experience of various laboratories.

— A staining method has been proposed to aim assessment.

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

Under certain climatic and environmental conditions, microorganisms can settle on and colonize the surface of plastics or plastics products. Their presence and/or their metabolic products might not only damage the plastic itself, but can also affect the serviceability of building materials and systems containing plastic parts.

The tests and test conditions specified in this document are empirical and cover most but not all potential applications.

For specific applications and for long-term tests, procedures which reflect performance under actual conditions are agreed upon.

The actions of microorganisms on plastics are influenced by two different processes.

a) Direct action: the deterioration of plastics which serve as a nutritive substance for the growth of the microorganisms.

b) Indirect action: the influence of metabolic products of the microorganisms, e.g. discolouration or further deterioration.

This document deals with both processes as well as their combined action.

Changes to the method are based on discussions among laboratories that have performed the test for at least 5 years. On an international level, discussions have taken place within the Plastic Group of the International Biodeterioration Research Group (IBRG) between scientists with extensive experience with this document as well as the testing of the interaction between microorganisms and plastics.

Plastics — Evaluation of the action of microorganisms

WARNING — Handling and manipulation of microorganisms which are potentially hazardous requires a high degree of technical competence. Only personnel trained in microbiological techniques should carry out such tests. Codes of practice for disinfection, sterilization and personal hygiene shall be strictly observed. It is recommended that workers consult IEC 60068-2-10 and ISO 7218.

1 Scope

This document specifies methods for determining the deterioration of plastics due to the action of fungi and bacteria and soil microorganisms. The aim is not to determine the biodegradability of plastics or the deterioration of natural fibre composites.

The type and extent of deterioration can be determined by

a) visual examination and/or

b) changes in mass and/or

c) changes in other physical properties.

The tests are applicable to all plastics that have an even surface and that can thus be easily cleaned. The exceptions are porous materials, such as plastic foams.

This document uses the same test fungi as IEC 60068-2-10. The IEC method, which uses so-called "assembled specimens", calls for inoculation of the specimens with a spore suspension, incubation of the inoculated specimens and assessment of the fungal growth as well as any physical attack on the specimens.

The volume of testing and the test strains used depend on the application envisaged for the plastic.

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 13934-1:2013, *Textiles — Tensile properties of fabrics — Part 1: Determination of maximum force and elongation at maximum force using the strip method*

EN 10088-1, *Stainless steels — Part 1: List of stainless steels*

EN 10088-2, *Stainless steels — Part 2: Technical delivery conditions for sheet/plate and strip corrosion resisting steels for general purposes*

EN 13697:2015, *Chemical disinfectants and antiseptics — Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas — Test method and requirements without mechanical action (phase 2, step 2)*

IEC 60068-2-10, *Environmental testing — Part 2-10: Tests — Test J and guidance: Mould growth*

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
biodeterioration
undesired change in the properties, such as colour, strength, mass, of a material due to the action of a microorganism

3.2
fungistatic effect
antimycotic effect of an antimicrobial treatment which prevents a given material from being overgrown by fungi under moist conditions

4 Principle

4.1 General

The test involves exposing test specimens of plastic to the action of selected test strains of fungi and bacteria (or, in the case of the soil-burial test, to microbially active soil) for specified or agreed periods of time under specified conditions of temperature and humidity.

At the end of the exposure, the test specimens are assessed before and/or after cleaning by visual examination and/or any change in mass or other physical properties is determined.

The results obtained with the specimens exposed to microorganisms (test series I) are compared with those obtained from retained reference specimens (test series 0) or sterile specimens (test series S) kept under the same conditions.

In the case of testing fungistatic properties, a visual assessment is made between test specimens free of biocides and with those containing biocides to demonstrate the effect of a biocide in a qualitative manner.

Short descriptions of the test methods used to determine the resistance of plastics to fungi (method A) or the fungistatic effects (method B), resistance to bacteria (method C) and resistance to soil microorganisms (method D) are given in 4.2 to 4.4.

4.2 Resistance to fungi

4.2.1 Method A: Fungal-growth test

Test specimens are exposed to a mixed suspension of fungal spores in the presence of a humidity ≥95 % relative humidity. After the limited nutrients from the spore itself are depleted through formation of a germination tube, the fungi can only grow at the expense of the material of the test specimens. If the specimens contain no nutritive component, the fungi cannot develop mycelia and there will be no deterioration of the plastic.

Method A is suitable for the assessment of the inherent resistance of plastics to fungal attack in the absence of other organic matter.

4.2.2 Method B: Determination of fungistatic effects

Test specimens are exposed to a mixed suspension of fungus spores in the presence of a complete nutrient medium, i.e. with a carbon source. Even if the plastic does not contain any nutritive elements, the fungi can grow over the specimens and their metabolic products can attack the material by metabolizing the nutrient-agar medium.

Any inhibition of growth either on the plastic or in the nutrient-agar medium (zone of inhibition) shows fungistatic activity of the plastic or the presence of a fungicidal treatment.

In order to show a basic qualitative effect of a biocide in a plastic material, specimens free from biocide shall be included in the test. Only if these biocide-free specimens show more growth than the specimens containing biocides can a qualitative indication of fungistatic or fungicidal efficacy be determined.

4.3 Method C: Resistance to bacteria

The action of bacteria on test specimens is assessed using an incomplete medium without a carbon source[1)]. If there is no growth in the agar surrounding the specimen, then the specimen does not contain any nutritive components.

If a material to be tested claims added functionality, such as a product with hygienic effects, the plastic material should be tested according to ISO 22196 which provides guidance for measuring the basic antibacterial performance of non-porous (plastic) materials that have been treated with a biocide with the intention of introducing antibacterial/hygienic properties into that material.

4.4 Method D: Resistance to microbially active soil (soil-burial test)

Test specimens are completely buried in natural soil with a known water-holding capacity and a specified moisture content (see Annex A).

The soil-burial test has been included in this document because many plastics are used in permanent contact with soil and exposed to high humidities.

4.5 Choice of properties for assessment of biodeterioration

The choice of the properties to be determined depends on the aim of the test. A visual assessment of biological attack shall always be made as the first stage in assessing the resistance of the plastic.

It is recommended that determinations be made of those properties which clearly indicate surface changes, such as surface gloss, flexural properties, impact resistance and hardness.

5 Apparatus and materials

5.1 For all tests

5.1.1 Incubators, capable of controlling the temperature to ±1 °C at 29 °C at a relative humidity of ≥ 95 %.

5.1.2 Oven, capable of controlling the temperature at 45 °C ± 1 °C for drying test specimens and at between 103 °C and 105 °C for determining the water-holding capacity of soil.

5.1.3 Climatic chamber, capable of maintaining standard temperature and humidity conditions (23 °C and 50 % R.H.) for the conditioning of test and control specimens.

5.1.4 Autoclave, capable of maintaining a temperature and pressure of 120 °C and 2 bar, respectively, for sterilizing glass containers or glass Petri dishes and soil.

5.1.5 Analytical balance, accurate to 0,1 mg.

5.1.6 Laboratory centrifuge.

1) Agar-agar used in media needs to be very low in carbon.

5.1.7 Stereoscopic microscope, magnification × 50.

5.1.8 Glass or plastic disposable Petri dishes with vented lids, of suitable size for exposing test specimens.

5.1.9 Glass containers with lid, with a volume of at least 1 l (height approximately. 16 cm; diameter approximately 11 cm), with sufficient space to allow a reservoir of water to be set-up below the Petri dishes.

5.1.10 Distilled or deionized water.

The water used for the preparation of all solutions and nutritive media and for all determinations shall be distilled or deionized and have a conductivity of < 1 µS/cm.

5.1.11 Microbicidal solution, is Ethanol-water mixture, in the proportions, by mass, of 70:30.

5.1.12 Stainless steel coupons (method A).

These shall be 1.4301 (in accordance with EN 10088-1) stainless steel discs (about 2 cm in diameter) with Grade 2 B (in accordance with the requirements of EN 10088-2) finish on both sides. The surface shall be as flat as possible and the stainless steel should have a gauge of 1,2 mm or 1,5 mm.

NOTE Suitable stainless steel discs can usually be purchased from local engineering companies.

5.1.13 Buchner funnel, with a sintered filter.

5.1.14 Grid (5 mm × 5 mm), inscribed on clear carrier (e.g. glass or plastic) to be used to evaluate the colonisation of the surface of test specimens by fungi.

5.1.15 *o*-phenylphenol solution.

Dissolve 1 g of *o*-phenylphenol in 50 ml of 90 % ethanol, make up to 1 000 ml with water and adjust the pH to 3,5 by adding lactic acid drop by drop.

5.2 For tests with fungi

5.2.1 Test fungi

The test fungi shall be obtained from national culture collections. The strains to be used are listed in Table 1, and shall be stated in the test report. Alternative culture collections and strain numbers of the same fungal strains are listed in Annex C.

Table 1 — Fungal strains to be used when testing plastics without electronic application

Name	Strain
Aspergillus niger	ATCC 6275
Penicillium pinophilum	ATCC 36839
Paecilomyces variotii	ATCC 18502
Trichoderma virens	ATCC 9645
Chaetomium globosum	ATCC 6205

If there are technical reasons, and by agreement between the interested parties, other species may be used. In this case, too, the strains used shall be stated in the test report.