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Plastics — Evaluation of the action of microorganisms

Plastiques — Évaluation de l'action des micro-organismes

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Contents

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

This second edition cancels and replaces the first edition (ISO 846:1978), which has been technically revised.

The Plastics Project Group of the IBRG (International Biodeterioration Research Group) carried out several interlaboratory tests between 1984 and 1990, using the 1978 edition of this standard, with the aim of checking the reproducibility of the test results. The experience gained from these tests has been incorporated in the present edition. In addition, a soil-burial test method has been included in subclause 8.5, based on a specification the Eidgenössische Materialprüfungsanstalt in St. Gallen, Switzerland.

Annex A forms an integral part of this International Standard. Annexes B and C are for information only.

Introduction

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

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

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

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 micro-organisms, e.g. discolouration or further deterioration.

This International Standard deals with both of these two processes, as well as their combined action.

Plastics — Evaluation of the action of microorganisms

WARNING — Handling and manipulation of microorganisms which are potentially hazardous requires a high degree of technical competence and may be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests. Codes of practice for disinfection, sterilization and personal hygiene must be strictly observed.

It is recommended that workers consult IEC 68-2-10:1988, appendix A "Danger to personnel", and ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

1 Scope

This International Standard 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.

The type and extent of deterioration may 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 articles made of plastic that have an even surface and that can thus be easily cleaned. The exceptions are porous materials, such as plastic foams.

This International Standard uses the same test fungi as IEC 68-2-10. 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 will depend on the application envisaged for the plastic. These parameters should therefore be agreed upon before the tests and should be stated in the test report.

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 291:—[1], *Plastics — Standard atmospheres for conditioning and testing.*

IEC 68-2-10:1988, *Basic environmental testing procedures — Part 2: Tests — Test J and guidance: Mould growth.*

3 Definitions

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

3.1 biodeterioration: A change in the chemical or physical properties of a material due to the action of a microorganism.

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

3.3 biodegradation: The term "*biodegradation*" is being discussed by TC 61/SC 5/WG 22, *Biodegradability*, and the official definition will be included here when it is available.

4 Principle

4.1 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 biological attack (batch I) are compared with those obtained from untreated specimens (batch 0) or sterile specimens (batch S) kept under the same conditions.

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

4.2.1 Resistance to fungi

4.2.1.1 Method A: Fungal-growth test

Test specimens are exposed to a mixed suspension of fungus spores in the presence of an incomplete nutritive medium (without a carbon source). The fungi can only grow at the expense of the material. If the specimens contain no nutritive component, the fungi cannot develop mycelia and there is no deterioration of the plastic.

1) To be published. (Revision of ISO 291:1977)

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

It is recommended that, when method A is carried out, methods B and B' are also carried out to assist in the interpretation of the results.

4.2.1.2 Methods B and B': Determination of fungistatic effects

Test specimens are exposed to a mixed suspension of fungus spores in the presence of a complete 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.

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

In method B', the specimens are not placed on the nutritive medium until it is completely overgrown.

Methods B and B' are used when surface contamination is expected. In order to save time, and for a better understanding of the phenomenon, it is recommended that the two methods are carried out simultaneously.

4.2.2 Method C: Resistance to bacteria

The action of bacteria on test specimens is assessed using an incomplete medium. If there is no growth in the agar round the specimen, then the specimen does not contain any nutritive components.

4.2.3 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 International Standard because many plastics are used in permanent contact with soil and exposed to high humidities.

4.3 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 should preferably always be made as the first stage in assessing the resistance of the plastic.

The measurement of changes in mass is recommended, especially for those plastics that contain biologically degradable substances, such as plasticizers, lubricants and stabilizers (as in plasticized PVC, for instance). The measured loss is, in this case, often lower than the actual loss as the biologically degradable substance is only partly utilized and the metabolic products often remain in the plastic.

When, above all, the surface is affected, 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

That used for tests involving fungal and bacterial attack shall be capable of controlling the temperature to ±1 °C at any temperature from 20 °C to 35 °C at a relative humidity of 90 % or greater.

That used for soil-burial tests shall be capable of controlling the temperature to ±1 °C at 29 °C at a relative humidity of 95% or greater.

NOTE — Experience has shown that it is preferable to use two incubators: one for Petri dish tests and another for soil-burial tests.

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

5.1.3 Desiccator, 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 Petri dishes and soil.

5.1.5 Analytical balance, accurate to 0,1 mg.

5.1.6 Laboratory centrifuge.

5.1.7 Stereoscopic microscope, magnification × 50.

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

5.1.9 Glass containers, with a volume of about 1 litre (height 16 cm, diameter 11 cm), for example preserving jars with covers.

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 solutions:

5.1.11.1 Ethanol-water mixture, in the proportions, by mass, of 70:30.

5.1.11.2 *o*-Phenylphenol.

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.

The microbicidal solution used shall be stated in the test report.

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 be stated in the test report.

Table 1

Name	Strain
Aspergillus niger van Tieghem	ATCC 6275
Penicillium funiculosum Thom	CMI 114933
Paecilomyces variotii Bainier	ATCC 18502
Gliocladium virens Miller *et al.*	ATCC 9645
Chaetomium globosum Kunze: Fries	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.

When carrying out tests on plastics intended for use in electronic components and electronic equipment, using the method specified in IEC 68-2-10, use *Aspergillus niger, Penicillium funiculosum, Paecilomyces variotii* and *Gliocladium virens* from table 1 and the four strains given in table 2.

Table 2

Name	Strain
Aspergillus terreus Thom	QM 82j
Aureobasidium pullulans (de Bary) Arnaud	ATCC 9348
Penicillium ochrochloron Biourge	ATCC 9112
Scopulariopsis brevicaulis (Saccardo) Bainier	CMI 49528

5.2.2 Stock strains.

Culture the test fungi (5.2.1) in tubes on agar slants of the following composition:

Oatmeal	20 g
Malt extract	10 g
Agar	20 g
Water	1 000 ml

Sterilize at 120 °C ± 1 °C for 20 min in an autoclave in an atmosphere saturated with water vapour.

After incubation at 29 °C ± 1 °C or 24 °C ± 1 °C, well sporulating cultures may then be used. They shall not be stored for more than 4 weeks at this temperature.

Because of the possibility of genetic and physiological changes in the test fungi during culturing on artificial media, the intervals between subculturing shall be reduced to a minimum by suitable measures (e.g. lyophilization of cultures, storage at + 4 °C or in liquid nitrogen).

5.2.3 Solutions and nutritive media:

5.2.3.1 Stock mineral-salt solution, of the following composition (use only chemicals of analytical grade or equivalent purity):

$NaNO_3$	2,0 g
KH_2PO_4	0,7 g
K_2HPO_4	0,3 g
KCl	0,5 g
$MgSO_4 \cdot 7H_2O$	0,5 g
$FeSO_4 \cdot 7H_2O$	0,01 g
H_2O	1 000 ml

Adjust the pH to 6,0 to 6,5 with sterile 0,01 mol/l NaOH solution.

5.2.3.2 Mineral-salt/wetting-agent solution, prepared by adding to 1 litre of stock mineral-salt solution (5.2.3.1) 0,1 g of a non-toxic wetting agent such as *N*-methyltaurine or polyglycol ether and sterilizing in an autoclave at 120 °C ± 1 °C for 20 min.

5.2.3.3 Mineral-salt/glucose solution, prepared by adding to stock mineral-salt solution (5.2.3.1) sufficient glucose to give a concentration of 30 g/l ± 1 g/l and sterilizing in an autoclave at 115 °C ± 1 °C for 30 min.

5.2.3.4 Incomplete agar medium, prepared by adding to stock mineral-salt solution (5.2.3.1) sufficient agar to give a concentration of 20 g/l. Dissolve the agar by boiling the solution whilst stirring. Sterilize in an autoclave at 120 °C ± 1 °C for 20 min. Adjust the pH to 6,0 to 6,5 with sterile 0,01 mol/l NaOH solution.

5.2.3.5 Complete agar medium, prepared by adding to the incomplete agar medium (5.2.3.4) sufficient glucose to give a concentration of 30 g/l ± 1 g/l. Sterilize in an autoclave at 115 °C ± 1 °C for 30 min. After sterilization, adjust the pH to between 6,0 and 6,5 at 20 °C with sterile 0,01 mol/l NaOH solution.

5.3 For tests with bacteria

5.3.1 Test bacterium *Pseudomonas aeruginosa*, strain NCTC 8060 or ATCC 13388.

A well-defined strain of the test bacterium shall be obtained from a national culture collection. Cultivate the test strain on brain-heart infusion agar (5.3.2.1).

If, by agreement, additional test bacteria are used, they shall be mentioned in the test report.

5.3.2 Nutritive media and solutions

5.3.2.1 Brain-heart infusion agar.

Casein soybean peptone agar may be used as an alternative.

The medium may be obtained from commercial suppliers and shall be prepared in accordance with the manufacturer's instructions.

5.3.2.2 Brain-heart infusion broth.

Casein soybean peptone broth may be used as an alternative.

The medium may be obtained from commercial suppliers and shall be prepared in accordance with the manufacturer's instructions.

5.3.2.3 Mineral-salt agar, prepared by making up a solution of the following composition:

KH_2PO_4	0,7 g
K_2HPO_4	0,7 g
$MgSO_4 \cdot 7H_2O$	0,7 g
NH_4NO_3	1 g
NaCl	0,005 g
$FeSO_4 \cdot 7H_2O$	0,002 g
$ZnSO_4 \cdot 7H_2O$	0,002 g
$MnSO_4 \cdot 7H_2O$	0,001 g
H_2O	1 000 ml

and adding 20 g of agar to the solution. Sterilize in an autoclave at 120 °C ± 1 °C for 20 min. Adjust the pH to 7,0 at 20 °C with 0,01 mol/l NaOH solution.

5.3.2.4 Sterile buffer solution, pH 7,0 at 20 °C.

Prepare the following two solutions separately:

KH_2PO_4	9,1 g/l	(solution A)
Na_2HPO_4	11,9 g/l	(solution B)

Mix 600 ml of solution A with 400 ml of solution B. Sterilize in an autoclave at 120 °C ± 1 °C for 20 min. Adjust the pH to 7,0 at 20 °C by adding 0,01 mol/l NaOH solution.

5.4 For soil-burial tests

Use an activated soil with a moisture content of (60 ± 5) % of the water-holding capacity of the soil (see annex A).

The water-holding capacity is the water content of a soil when it is saturated with water.

The pH of an aqueous soil extract (1 g of soil in 20 g of water) shall be between 4,0 and 7,0.

Determine the moisture content and water-holding capacity of the soil in accordance with annex A. If the moisture content of the soil exceeds the above figure, spread it out in a thin layer under ambient laboratory conditions. Do not heat the soil or allow it to dry out as this may affect the soil microflora. If the moisture content needs to be raised, use an aqueous solution of 1 g of ammonium nitrate and 0,2 g of dipotassium phosphate in 1 litre of water.

6 Test specimens

6.1 Shape and dimensions

The shape and dimensions of the specimens will depend on any tests to be carried out following exposure to the fungi, bacteria or soil.