INTERNATIONAL STANDARD 846

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Plastics — Determination of behaviour under the action of fungi and bacteria — Evaluation by visual examination or measurement of change in mass or physical properties

Plastiques — Détermination du comportement sous l'action des champignons et des bactéries — Évaluation par estimation visuelle ou par mesurage des variations de masse ou de caractéristiques physiques

First edition — 1978-09-01

UDC 678.5/.8 : 620.193.81/.82

Ref. No. ISO 846-1978 (E)

Descriptors : plastics, physicochemical tests, pest resistance tests, bacteria, fungi, biodeterioration, visual inspection, ponderal stability, test specimen conditioning.

Price based on 10 pages

FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 846 was developed by Technical Committee ISO/TC 61, *Plastics*, and was circulated to the member bodies in December 1976.

It has been approved by the member bodies of the following countries :

Austria	India	Portugal
Belgium	Iran	Spain
Brazil	Israel	Sweden
Canada	Korea, Rep. of	Switzerland
Chile	Mexico	Turkey
Czechoslovakia	Netherlands	U.S.A.
France	New Zealand	
Hungary	Poland	

The member bodies of the following countries expressed disapproval of the document on technical grounds :

Australia
United Kingdom

This International Standard cancels and replaces ISO Recommendation R 846-1968, of which it constitutes a technical revision.

Printed in Switzerland

Plastics — Determination of behaviour under the action of fungi and bacteria — Evaluation by visual examination or measurement of change in mass or physical properties

0 INTRODUCTION

The action of micro-organisms on plastics can be divided into two processes which take place simultaneously in most cases.

In the first process the action results from the ability of micro-organisms to grow on the plastic material itself (as the sole carbon source) and consequently cause changes.

In the second process the action is caused by the metabolic products of the micro-organisms which have developed on the plastic material or on surface contamination when the plastic material has no antimicrobial effect.

This International Standard deals with the combined action of these two processes.

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies methods for determining the behaviour of plastics under the action of fungi or bacteria, by visual examination and/or by measurement of the change in mass and/or physical properties.

It is applicable to all plastics except materials where complete cleaning is impracticable, for example cellular materials.

2 REFERENCES

ISO 75, *Plastics and ebonite — Determination of temperature of deflection under load.*

ISO 178, *Plastics — Determination of flexural properties of rigid plastics.*

ISO/R 179, *Plastics — Determination of the Charpy impact resistance of rigid plastics (Charpy impact flexural test).*

ISO/R 180, *Plastics — Determination of the Izod impact resistance of rigid plastics (Izod impact flexural tests).*

ISO 291, *Plastics — Standard atmospheres for conditioning and testing.*

ISO 527, *Plastics — Determination of tensile properties.*[1)]

ISO 537, *Plastics — Testing with the torsion pendulum.*[1)]

ISO 604, *Plastics — Determination of compressive properties.*

ISO 868, *Plastics — Determination of indentation hardness by means of a durometer (Shore hardness).*

ISO/R 974, *Plastics — Method of determining the brittleness temperature by impact.*

NOTE — By agreement between the interested parties, properties specified in other International Standards may be determined where they are judged to be more relevant to the use of the plastics.

3 PRINCIPLE

The test consists in exposing test specimens of plastics to the action of fungi or bacteria for specified periods of time and in specified conditions of temperature and humidity.

At the end of the exposure, the test specimens are evaluated visually, both before and after cleaning, and/or tested by weighing and/or measuring physical properties.

These test results are compared with those obtained on unexposed test specimens, i.e. specimens kept

- either under normal conditions;
- or under the same conditions as for the microbiological exposure except that they are sterile.

The test for resistance to fungi can be carried out by two methods :

a) In method A, the plastic specimen is exposed to spores of specified fungi which are supplied only with an incomplete nutritive medium. The fungi can only grow at the expense of the components of the plastics. If there is no nutritive component or contamination, the spores do not grow and there is no attack. The test carried out by this method is therefore sometimes called "test for growth".

b) In method B, the plastic specimen is exposed to spores of specified fungi which are distributed on a complete nutritive medium which permits their growth. Even if the plastic material does not supply any nutritive element, the fungi can grow over the specimen and their secreted metabolic products attack the substrate.

1) At present at the stage of draft. (Revision of ISO Recommendation.)

On the other hand, an inhibition of the growth shows the fungistatic activity of the plastic. Consequently, the test according to this method is sometimes called "test for fungistatic effect".

The test for resistance to bacteria consists in exposing the plastic specimen to the action of bacteria on an incomplete nutritive medium.

4 CHOICE OF EXPOSURE METHOD AND OF CHARACTERISTICS TO BE DETERMINED

4.1 Choice of exposure method

The choice of one or several methods of exposure depends on the proposed use of the plastic.

For example, it can be stated that for the determination of the resistance to fungi, method A is sufficient when the conditions of use of the plastic exclude any contamination of the surface by organic substances. When the conditions of use suppose heavy contamination, method B is recommended.

NOTE – To save time and gain a better understanding of the whole phenomenon, the two tests should be made simultaneously.

4.2 Choice of characteristics to be determined before and after exposure

The visual examination after exposure can always be carried out, and because of the simplicity of the method, this examination is usually recommended. The choice of the properties to be measured depends essentially, as does the choice of the exposure method, on the proposed use of the plastic.

However, this choice can also depend on the composition of the plastic.

For example, determination of change in mass is specially suitable for plastics containing biodegradable components such as plasticizers, lubricants, stabilizers (for example plasticized PVC).

NOTES

1 In this last case, the loss in mass is often less than the actual loss of plasticizer, due to the fact that the biodegradable component is only partially degraded and that the metabolic residues remain inside the plastic.

2 As the attack on plastics occurs on the surface, it is often useful to determine surface properties, for example tensile stress at small elongations, flexural modulus, etc.

5 MATERIALS, REAGENTS, SOLUTIONS AND MEDIA

Water used for the preparation of media and solutions as well as for tests shall be distilled water or water of similar purity with a minimum resistivity of 1 MΩ·cm.

Because of pathogenic hazards of fungi and bacteria, it is essential that the handling of micro-organisms be carried out by staff qualified in microbiology. In general, the normal safety regulations for microbiological laboratories are to be observed.

5.1 FOR ALL TESTS

5.1.1 **Mercury(II) chloride,** 1 g/l aqueous solution

WARNING – Mercury(II) chloride is very toxic. Avoid inhalation or contact with the skin. It is recommended that the solution should not be sprayed but should be applied by means of a syringe or safety pipette. A mouth pipette should not be used.

5.2 FOR TESTS WITH FUNGI

5.2.1 Fungi for tests

5.2.1.1 *List of fungi*

Name	**Reference of typical culture collection** (for guidance only)
Aspergillus niger Van Tieghem	ATCC 6275
Penicillium funiculosum Thom	IAM 7013, CMI 114933
Paecilomyces variotii Bainier	IAM 5001, ATCC 10121
Trichoderma viride Pers. ex Fr.	IAM 5061, ATCC 9645
Chaetomium globosum Kunze	ATCC 6205

NOTE – If there are special technical reasons, and by agreement between the interested parties, additional species may be used. The species used shall be stated in the test report. In order to perform tests on plastics intended for use in electronic components and electronic equipment in accordance with IEC Publication 68-2-10, *Basic environmental testing procedures – Test J : Mould growth*, use the above fungi, with the exception of *Chaetomium globosum* Kunze, and the following additional species : *Aspergillus terreus*, Thom, PQMD 82 j, *Aureobasidium pullulans*, (De Bary) Arnaud, ATCC 9348, *Penicillium ochro-chloron*, Biourge, ATCC 9112, *Scopulariopsis brevicaulis*, (Sacc.) Bain Va. glabra, Thom, IAM 5146.

5.2.1.2 *Origin of test strains*

The test fungi used shall be well-defined strains obtained from official biological centres. The references of strains must be noted and stated in the test report.

NOTE – The addresses of official biological centres can be obtained from national standards associations in each country.

5.2.1.3 *Stock cultures*

The test strains (5.2.1.1) can be maintained in test tubes on the following medium :

oatmeal :	20 g
malt extract :	10 g
agar :	20 g
water :	1 000 ml

Sterilize at 120 ± 1 °C for 20 min in an autoclave.

The cultures can be used after incubation at 25 ± 2 °C and should not be stored for more than 1 month at this temperature.

In order to maintain the stock culture in good condition, it is necessary to subculture each species at least every 4 weeks.

5.2.2 Solutions and media for tests with fungi

Their preparation is given in the annex. These solutions and media are :

- basic mineral salts solution (A.1.1);
- mineral salts wetting solution (A.1.2);
- glucose mineral salts wetting solution (A.1.3);
- incomplete agar medium (A.1.4);
- complete agar media (A.1.5).

5.3 FOR TESTS WITH BACTERIA

5.3.1 Test bacteria

5.3.1.1 *Bacterium*

Pseudomonas aeruginosa — strain NCTC 8060 or ATCC 13888.

NOTE — Another strain may be used; if so, it shall be stated in the test report.

5.3.1.2 *Origin of the strain*

The bacterium or bacteria must be well-defined strains obtained from official biological centres (see note in 5.2.1.2).

5.3.1.3 *Stock culture*

Cultivate the test strains on brain-heart infusion agar (see A.2.1).

5.3.2 Solutions and media for tests with bacteria

Their preparation is given in the annex. These solutions and media are :

- brain-heart infusion agar (A.2.1);
- brain-heart infusion (A.2.2);
- mineral salts agar (A.2.3);
- sterile buffered solution pH 7,0 at 20 °C (A.2.4).

6 APPARATUS

Ordinary microbiological laboratory apparatus (autoclaves, ovens, incubators, centrifuges, stereoscopic microscope with a magnification of about 50 X) and in particular :

6.1 Test containers

The use of Petri dishes 100 to 120 mm in diameter is recommended.

7 TEST SPECIMENS

7.1 Dimensions

The dimensions of test specimens depend on the tests to be made after exposure to the micro-organisms.

Concerning thickness, if the behaviour of a sheet is to be studied, test specimens should be cut out of this sheet and so have the same thickness. On the other hand, if the behaviour of the material itself is to be studied, it is recommended that test specimens less than 0,5 mm thick be taken.

Concerning other dimensions, the following recommendations apply :

a) for visual evaluation, use square test specimens with sides of 30 to 40 mm and a thickness of approximately 2 mm;

b) for measuring change in mass, use 10 mm x 40 mm test specimens (note that to facilitate the comparison, all the test specimens corresponding to the same series of tests should have the same dimensions);

c) for measuring changes in physical properties, use the dimensions of test specimens that are specified in the International Standards concerning the determination of these properties.

7.2 Number

For each sample and each method to be used [i.e. method A (see 8.2), method B (see 8.3) and method B′ (see note to 8.3.6)], prepare three groups of test specimens.

One group will be stored in the laboratory (zero control, group O), a second group will be exposed to micro-organisms (inoculated specimens, group I), and the third group will be exposed to the same conditions of incubation as the previous ones but not inoculated (sterile incubated control, group S).

a) For the visual examination, prepare at least three test specimens per group, i.e. a minimum of nine test specimens.

b) For each one of the other properties studied, the number of test specimens per group is stated in the relevant International Standard (see clause 2). In particular, in the case of the determination of change in mass, this number is six, so that eighteen test specimens shall be prepared.

NOTE — In general, all the determinations are performed separately, but of course the specimens for determination of change in mass or in mechanical properties may be used for the visual examination.

7.3 Cleaning

Dip the specimens to be tested by method A and those methods using bacteria for about 1 min in 70 % (*V/V*) ethanol and dry them in air. Avoid consequent contami-

nation with greasy fingerprints and organic matter by handling them with forceps.

NOTE – Cleaning with ethanol should not be carried out if this solvent attacks the material to be tested; test specimens for method B and method B′ should not be cleaned with ethanol.

7.4 Labelling and storing

Label the test specimens in a convenient manner, avoiding contamination.

Store the test specimens in a closed container.

7.5 Conditioning and weighing

Dry in a desiccator the three groups of test specimens (O, I, S) to be used for determination of change in mass, until the mass of each test specimen is constant to the nearest 0,1 mg (usually 48 h). Record the mass of each test specimen (m_1, m_2, etc.).

The groups of test specimens for the visual examination and/or for determination of the changes in physical properties do not need conditioning at this stage.

8 TEST PROCEDURES

8.1 Choice of procedure and of characteristics to be measured – General scheme of test procedures

The choice of the test procedure and of the properties to be measured chiefly depends on the conditions of use of the material under test but also depends on the plastic itself (see clause 4).

Table 1 gives the general scheme of test procedures for the different methods.

8.2 Procedure with fungi by method A (Test for growth)

8.2.1 *Filling the test containers*

Prepare sufficient test containers (6.1) to hold the required number of test specimens. Heat the incomplete agar medium (A.1.4) to liquefy it and, under aseptic conditions, pour a sufficient amount into each container to give a depth of between 5 and 10 mm. Allow to cool to solidify.

8.2.2 *Arrangement of test specimens*

Place as many specimens as possible flat on the solidified medium, avoiding any contact of the specimens with one another or with the walls of the container and ensure that equal numbers of specimens are in each container.

Randomly divide the prepared containers into two equal groups, one labelled I, the other S.

8.2.3 *Preparation of the inoculum spore suspension*

See clause A.3 of the annex for the preparation of the spore suspension in the solution (A.1.2).

8.2.4 *Viability control of the spores*

Fill two test containers (6.1) with complete agar medium (A.1.5) according to the procedure given in 8.2.1. Inoculate them by spraying the spore suspension (A.3.2) on the agar surface.

Incubate at 29 ± 1 °C for 3 to 4 days. In the absence of copious growth, prepare a new spore suspension from other culture tubes and use it to repeat the whole test.

TABLE 1 – **General scheme of test procedures**

		Tests with fungi							**Tests with bacteria**	
		Method								
		A		**B**		**B′**				
Reference to sub-clause		8.2		8.3		Note in 8.3.6			8.4	
Filling of containers		Incomplete agar medium (A.1.4)		Complete agar medium (A.1.5)		None	Complete agar medium (A.1.5)		Mineral salts agar medium inoculated (8.4.5)	Mineral salts agar (8.4.2)
Group of test specimens		I	S	I	S	I	I	S	I	S
Solution sprayed on test specimens		Spore suspension (8.2.3)	$HgCl_2$ (5.1.1)	Spore suspension (8.3.3)	$HgCl_2$ (5.1.1)	Spore suspension (8.3.3)		$HgCl_2$ (5.1.1)	None	$HgCl_2$ (5.1.1)
Incubation	**Conditions**	Temperature : 29 ± 1 °C and relative humidity : > 90 %								
	Time	4 weeks or more								

8.2.5 *Inoculation or disinfection of test specimens*

On each test specimen (7.2) in the group I containers and on the agar surface, spray or pipette evenly 0,1 ml of inoculum spore suspension (A.3.2).

On each test specimen in the group S containers and on the agar surface, pipette the aqueous solution of mercury(II) chloride (5.1.1).

8.2.6 *Incubation*

Close the test containers and incubate both the inoculated test specimens and the sterile controls at 29 ± 1 °C and > 90 % relative humidity for 4 weeks or longer by agreement between the interested parties.

Precautions shall be taken to prevent water dropping onto the surface.

If the test lasts more than 4 weeks, a supplementary series of test specimens shall be used.

NOTE – If the fungal growth is clearly visible to the naked eye during the 4 week incubation period, the test may be considered as completed if only the visual examination is required.

8.3 Procedure with fungi by method B (Test for fungistatic effect)

8.3.1 *Filling the test containers*

Follow the instructions given in 8.2.1 but using the complete agar medium (A.1.5).

8.3.2 *Arrangement of test specimens*

Follow the instructions given in 8.2.2, except that cleaning of test specimens is not necessary (see note in 7.3).

8.3.3 *Preparation of the spore suspension for inoculation*

See clause A.3 of the annex for the preparation of the spore suspension in the solution (A.1.3).

8.3.4 *Viability control of the spores*

Follow the instructions given in 8.2.4.

8.3.5 *Inoculation or disinfection of test specimens and medium*

On each test specimen (7.2) in group I containers and on the agar surface, spray or pipette the aqueous inoculum spore suspension (see 8.3.3 and clause A.3 of the annex).

On each test specimen in the group S containers and on the agar surface, pipette the aqueous solution of mercury(II) chloride (5.1.1).

8.3.6 *Incubation*

Close the test containers and incubate both the inoculated test specimens (group I) and the sterile controls (group S) at 29 ± 1 °C and > 90 % relative humidity for 4 weeks.

It can be agreed between the interested parties that incubation should be longer, for example 8 weeks. In this case, it is necessary after each period of 4 weeks to spray or pipette onto the test specimens a small quantity of glucose/mineral salts solution (A.1.3) in order to maintain the growth of fungi.

NOTE – **Method B'**

A possible variant of method B is to wait until the fungi are well established but not fruiting before placing the test specimens in the containers.

For this method, follow the instructions given in 8.3.1, but in 8.3.2 place only half of the test specimens in the test containers filled with agar. These constitute the group S.

The other half of the test specimens are placed in unfilled test containers (6.1).

Thus there is one group of containers filled with agar and test specimens constituting group S, and a second group of containers filled with agar, constituting, together with the third group of containers with test specimens only, group I.

Follow the instructions given in 8.3.3, 8.3.4 and 8.3.5 but inoculate all group I containers and disinfect the group S samples.

Incubate the three groups of containers at 29 ± 1 °C and > 90 % relative humidity.

Follow visually the growth of the culture in the group I containers. As soon as this is well established but not fruiting (after about 2 to 3 days), remove the test specimens from the unfilled containers and place them on the cultures.

Re-incubate in the same conditions and for the same period of time as given in 8.3.6.

This variant of method B generally corresponds to a more severe attack.

8.4 Procedure with bacteria

8.4.1 *Sterilization of the test specimens*

Test specimens are sterilized by a suitable means. Methods used in laboratories are autoclaving at 120 ± 1 °C for 20 min or, when heating cannot be tolerated, fumigating with an appropriate agent and ventilating well under aseptic conditions to remove all fumes prior to carrying out the test.

Store the test specimens in a sterile container and thereafter handle them with sterile forceps.

NOTE – Carefully follow the safety instructions of the supplier of the fumigant.

8.4.2 *Preparation of mineral salts agar medium*

Prepare a sufficient amount for the proposed use, following the instructions given in A.2.3.

Allow to cool to 45 °C and proceed as stated in 8.4.5.

8.4.3 *Preparation of bacterium cell suspension*

From the bacterium stock culture on agar medium (5.3.1.3), inoculate brain-heart broth medium (A.2.2) and incubate for 24 h at 29 ± 1 °C. With a sterile platinum loop, transfer from the latter to 10 ml of sterile buffer solution (A.2.4).

Dilute this suspension with solution A.2.4 to obtain a cell suspension containing about 10^6 cells per millilitre as determined with a counting chamber or by turbidimetry.

This cell suspension shall be used within 1 h of preparation.

8.4.4 *Viability control*

At the same time as the test of the plastic, prepare two containers holding 10 ml of sterile brain-heart infusion agar (A.2.1). Add 3 drops of final cell suspension (8.4.3) and incubate for 24 to 48 h at 29 ± 1 °C. There should be copious growth of the bacterium in both containers; if not, repeat the whole test.

8.4.5 *Preparation of inoculated mineral salts agar medium*

At about 45 °C, inoculate the melted agar (8.4.2) with a sufficient amount of bacterial cell suspension to obtain a concentration of about 50 000 cells per millilitre of agar, and mix.

8.4.6 *Filling of test containers and placing of test specimens*

Pour a sufficient amount of inoculated agar (8.4.5) into sterile test containers to provide an agar layer about 1 cm deep.

After the agar has solidified, place a first group of test specimens on the surface of the agar. Pour onto the test specimens sufficient inoculated agar (8.4.5) to cover them, and allow to gel (inoculated test specimens).

In the same way, pour into other sterile test containers an amount of uninoculated agar (8.4.2) and allow to solidify. Place a second group of test specimens on the agar and disinfect their surface with an aqueous solution of mercury(II) chloride (5.1.1) applied by means of a safety pipette. Pour onto the test specimens sufficient agar (8.4.2) to cover them, and allow to gel (sterile control test specimens).

8.4.7 *Incubation*

Close the test containers, and incubate both inoculated test specimens (group I) and sterile test specimens (group S) at 29 ± 1 °C and > 90 % relative humidity for 4 weeks, or longer by agreement between the interested parties.

NOTES

1 If the bacterial growth is clearly visible to the naked eye during the 4 week incubation period, the test may be considered as completed if only the visual examination is required.

2 For certain bacteria, another incubation temperature may be selected by agreement between the interested parties.

9 EXAMINATION OF TEST SPECIMENS

9.1 Examination of the growth of fungi or bacteria on test specimens for visual evaluation

The exposed test specimens (inoculated I and sterile S) are first inspected by the naked eye and then, if necessary, with a stereoscopic microscope (with a magnification of about 50 X).

The intensity of fungal growth is rated as follows :

9.1.1 *Method A*

0 — no growth apparent under the microscope;

1 — growth invisible or hardly visible to the naked eye, but clearly visible under the microscope;

2 — growth on the surface visible to the naked eye but not covering more than 25 % of the test specimen surface;

3 — growth on the surface visible to the naked eye and covering more than 25 % of the test specimen surface.

9.1.2 *Method B*

0 — no growth apparent even under the microscope. Inhibition zone absent or present; if present, the dimensions, in millimetres, shall be stated;

1 — growth invisible or hardly visible to the naked eye, but clearly visible under the microscope;

2 — slight growth covering less than 25 % of the test specimen surface;

3 — medium growth covering 25 to 50 % of the test specimen surface;

4 — considerable growth covering more than 50 % but less than 100 % of the specimen surface;

5 — heavy growth covering the entire specimen surface.

NOTES

1 If the visual observations on test specimens of the same group vary over a range of two scale ratings or more, it is recommended that the test be repeated, using other test specimens from the same sample.

2 In some cases the rating of specimens after cleaning (see 9.2.1) gives useful information; colour photography is an aid to recording visual inspection.

3 If there is no apparent growth, re-incubate the specimens for a further period of 4 to 8 weeks and re-examine them.

9.2 Examination of the test specimens intended for determination of change of mass and/or in physical property

9.2.1 *Cleaning of exposed test specimens (inoculated I and sterile S)*

Vessels with visual growth shall be inactivated either by gas sterilization or by autoclaving after the end of the test.

Remove the test specimens from cultures and media.

Dip them for 5 min in the solution of mercury(II) chloride (5.1.1).

Clean them under running water while gently rubbing between gloved fingers.

Wipe them with filter paper.

Allow to dry overnight in the air at room temperature.

9.2.2 *Weighing or conditioning of test specimens*

a) For determination of change in mass, place the three groups of specimens (controls O, inoculated I and sterile S) in a desiccator and weigh them regularly to the nearest 0,1 mg until constant mass is attained for each test specimen (in general 48 h). Let these masses be m'_1, m'_2, etc.

b) For determination of changes in physical properties, condition the three groups of test specimens (O, I, S) according to the requirements of ISO 291.

9.2.3 *Determination of change in mass*

Determine, for each test specimen, the difference in mass before (see 7.5) and after (see 9.2.2) exposure ($m'_1 - m_1$; $m'_2 - m_2$; etc.). This difference is generally negative, corresponding to a loss.

9.2.4 *Determination of changes in physical properties*

Determine, for each group (O, I, S), the values of the chosen physical property according to the appropriate International Standard for each determination.

The following properties may be determined :

- temperature of deflection under load (see ISO 75);
- flexural properties (see ISO 178);
- impact resistance (see ISO/R 179 and ISO/R 180);
- tensile properties (see ISO 527);
- torsional properties (see ISO 537);
- compressive properties (see ISO 604);
- indentation hardness (see ISO 868);
- brittleness temperature by impact (see ISO/R 974);
- etc.

10 CALCULATION AND EXPRESSION OF RESULTS

10.1 Visual evaluation

The results of visual observation on each individual test specimen shall be recorded.

The results shall be expressed for each test specimen in terms of the intensity of growth.

In practice, the intensity of growth corresponds to the evaluations in table 2.

TABLE 2 – **Evaluation**

Method	Intensity of growth	Evaluation of test material
A	0	The material is not a nutritive medium for micro-organisms (it is inert or fungistatic)
	1	The material contains nutritive substances or is contaminated to such a small degree that it permits only a slight growth
	2 or 3	The material is not resistant to a fungal attack and contains nutritive substances suitable for the development of micro-organisms
B or B'	0	Strong fungistatic effect
	0 + dimension of zone of inhibition	Strong fungistatic effect due to diffusing substance
	1	Fungistatic effect not quite complete
	2 to 5	Decreasing effectiveness, down to complete absence of fungistatic effect

NOTE – Whenever the visual examination of test specimens after cleaning (considered in note 2 in 9.1.2) is carried out, state the results obtained.

10.2 Change in mass

Calculate the average of the change in mass for each group of test specimens (O, I, S), i.e. the averages of values $(m' - m)$, namely

$\overline{\Delta m_O}$, $\overline{\Delta m_I}$, $\overline{\Delta m_S}$ (generally negative values : losses).

Calculate to the first decimal place the average percentage change in mass due to biological action according to the formula

$$\frac{[\overline{\Delta m_I} - (\overline{\Delta m_S} + \overline{\Delta m_O})] \times 100}{\overline{m_e}}$$

where

m_I is the mean of the masses of the inoculated specimens;

m_S is the mean of the masses of the sterile specimens;

m_O is the mean of the masses of the control specimens;

$\overline{m_e}$ is the mean of the original masses of the specimens.

10.3 Change in physical properties

Calculate, for each property, the average of the results for each group O, I, S, namely $\overline{\nu_O}$, $\overline{\nu_I}$, $\overline{\nu_S}$.