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Building and civil engineering sealants — Assessment of the fungal growth on sealant surfaces

Mastics pour le bâtiment et le génie civil — Évaluation de la croissance fongique à la surface des mastics

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Contents

Page

Foreword

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This document was prepared by Technical Committee ISO/TC 59, *Buildings and civil engineering works*, Subcommittee SC 8, *Sealants*.

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

Sealants used in high humidity environments experience growth of microorganisms; and it can be necessary to test the function of sealant under normal service conditions to ensure that the sealant surface remains free of the growth microorganisms over a designated function period. This test is designed to evaluate the 5 most common strains of microorganisms found on sealant surfaces.

Building and civil engineering sealants — Assessment of the fungal growth on sealant surfaces

1 Scope

This document specifies a method for the evaluation of the fungal growth on sealants which are used in joints in building construction.

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 6927, *Building and civil engineering sealants — Vocabulary*

ISO 846, *Plastics — Evaluation of the action of microorganisms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6927 and ISO 846 apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Apparatus

4.1 Incubators, used for tests involving fungal attack, shall be capable of controlling the temperature to ±1 °C at any temperature from 20 °C to 35 °C. A relative humidity of 90 % or greater one needs to be ensured during incubation.

4.2 Oven, capable of controlling the temperature at (45 ± 2) °C for drying test specimens.

4.3 Water bath or ventilated oven, capable of being controlled at (50 ± 1) °C for heating the specimen during the water immersion.

4.4 Autoclave, capable of maintaining a temperature and pressure of (120 ± 2) °C and 2 bar, respectively, for sterilizing Petri dishes.

4.5 Analytical balance, accurate to 0,1 mg.

4.6 Laboratory centrifuge.

4.7 Stereoscopic microscope, magnification × 50.

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

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

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

4.11 Glass containers, with a volume of about 2 000 ml. The containers shall have a cover to avoid evaporation.

4.12 Anti-adherent, PE or Polytetrafluoroethylene (PTFE) with thickness of (2 ± 0,5) mm and a width of minimum (50 ± 4) mm.

5 Test fungi preparation

5.1 Test fungi

The test fungi shall be obtained from national culture collections. The strains to be used are listed in Table 1.

Table 1 — Test fungi strains

Name	Strain
Fusarium oxysporum	ATCC 7808 or other corresponding national strain (e.g. CBS 267,50, DSM 841)
Aspergillus niger	ATCC 6275 or other corresponding national strain
Phoma herbarum	ATCC 12569 or other corresponding national strain (e.g. IMI 49948)
Exophiala jeanselmei	ATCC 34123 or other corresponding national strain (e.g. CBS 507,90, CBS 664,76, Duke 2405, IFM 4852, IHM 283, NCPF 2439)
Penicillium chrysogenum	ATCC 10106 or other corresponding national strain (e.g. CBS 306,48, IMI 24314)

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

5.2 Stock strains

Culture the test fungi (5.1) in tubes on agar slants of the following composition:

— Oatmeal 20 g

— Malt extract 10 g

— Agar 20 g

— Water 1 000 ml

Adjust the pH to 5,5 ± 0,2 with 0,01 mol/l HCl solution. Sterilize the agar composition at (120 ± 2) °C for 20 min in an autoclave in an atmosphere saturated with water vapour.

After incubation at (29 ± 1) °C, well sporulating cultures may then be used. They shall not be stored 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. lyophilisation of cultures, storage at +4 °C or in liquid nitrogen).

5.3 Solutions and nutritive media

5.3.1 Stock mineral-salt solution is 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

— $MgSO_4 7{\cdot}H_2O$ 0,5 g

— $FeSO_4 7{\cdot}H_2O$ 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.

5.3.2 Mineral-salt/wetting-agent solution is prepared by adding to 1 l of stock mineral-salt solution (5.3.1) 0,1 g of a non-toxic wetting agent such as N-methyltaurine or polyglycol ether and sterilizing in an autoclave at (120 ± 2) °C for 20 min.

5.3.3 Mineral-salt/glucose solution is prepared by adding to stock mineral-salt solution (5.3.1) sufficient glucose to give a concentration of 30 g/l and sterilizing in an autoclave at (120 ± 2) °C for 20 min.

5.3.4 Complete agar medium is prepared by adding to mineral-salt/glucose solution (5.3.3) sufficient agar to give a concentration of 20 g/l. Adjust the pH to between 6,0 and 6,5 at 20 °C with NaOH solution. Sterilize in an autoclave at (120 ± 2) °C for 20 min.

6 Preparation of test specimens

6.1 General

The sealant and the used materials (substrates and anti-adherent) shall be brought to (23 ± 2) °C over 24 h minimum.

6.2 Preparation and conditioning

10 or 20 test specimens shall be prepared. Extrude the sealant on a suitable anti-adherent substrate (e.g. PE or Polytetrafluoroethylene (PTFE)) and prepare a sheet with a thickness of (2 ± 0,5) mm and a width of minimum (50 ± 4) mm and a length (500 ± 10) mm for 10 test specimens.

The following precautions shall be taken:

a) the formation of air bubbles shall be avoided;

b) the sealant shall be pressed on surface of the anti-adherent substrate;

c) the sealant surface shall be tooled so that a flat surface is obtained.

After preparation, the strap shall be preconditioned for 28 days at (23 ± 2) °C and (50 ± 10) % relative humidity.

Cut 10 or 20 test specimens (Table 2) from the cured strap while ensuring the exposed surface (top surface) is the testing surface. The test specimens shall have the dimensions (50 ± 4) mm × (50 ± 4) mm × (2 ± 0,5) mm.

Table 2 — Test specimens for four groups

Items	Immersion	Without immersion
Solidified agar medium	5 specimens	5 specimens
Without solid medium (Optional)	5 specimens	5 specimens

6.3 Cleaning, labelling and storage of specimens

6.3.1 Cleaning

Dip the specimens into an ethanol-water mixture (4.10) for 1 min. Let the specimen dry. Carry out all subsequent handling of the specimens using forceps to avoid contamination by extraneous matter.

6.3.2 Labelling and storage

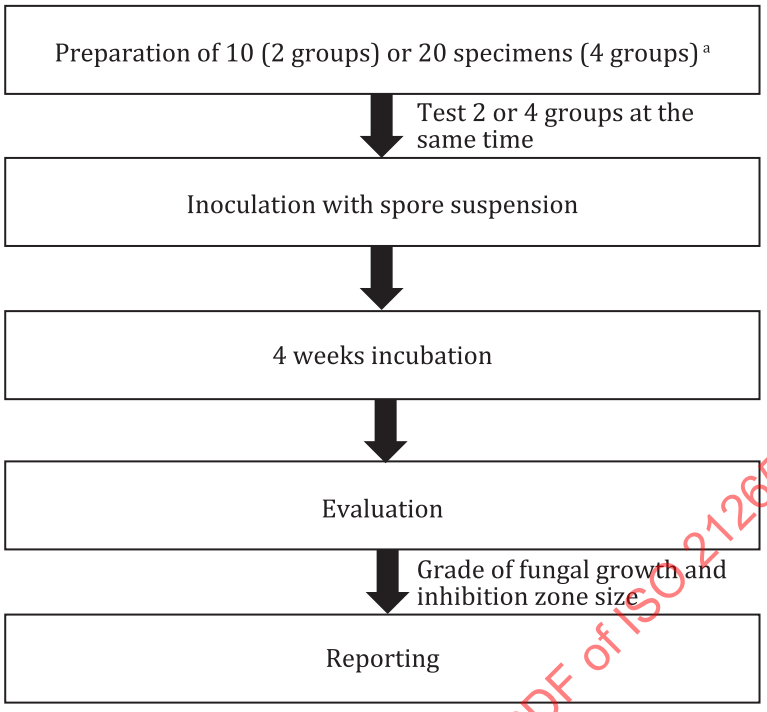
Store the cleaned specimens at ambient temperature.

It is advisable to store the specimen in suitable containers (e.g. Petri dishes) and label the Petri dishes, not the specimens, to avoid surface reactions.

7 Test procedures

7.1 General

Figure 1 shows the general procedure.



[a] See Table 2.

Figure 1 — Main procedure diagram

7.2 Test set-up and inoculation of test specimens

7.2.1 Specimens with or without water immersion

5 or 10 specimens prepared in accordance with 6.2 are stored in aseptic conditions during the time of immersion of 5 or 10 other specimens.

Single square or strap specimen which is prepared in accordance with 6.2 is used for the water immersion (Figure 2).

A container (volume about 2 000 ml) with (1 000 ± 20) ml deionized water can be used and square or strap specimen can be immersed in water. The square or strap needs to be fixed in place in a way that at least 90 % of the surface is exposed to water.

The containers shall be covered during the water immersion to avoid evaporation. During 28 days at (50 ± 1) °C, change the water 4 times per week. The change cycle should be longer than 24 h. Then the square or strap is removed from the containers and is conditioned at (23 ± 2) °C and (50 ± 10) % relative humidity for 24 h.

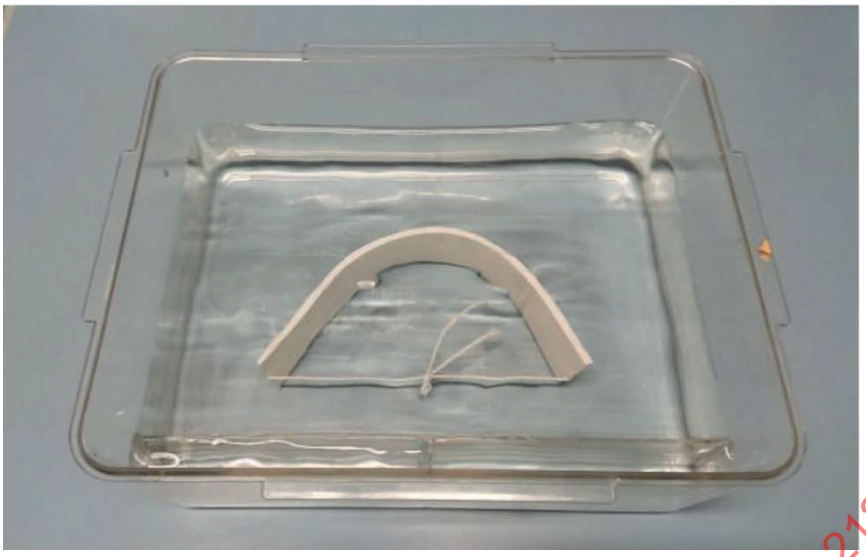


Figure 2 — Example of immersion of the test specimen

7.2.2 Test with solid medium: filling the petri dishes

After sterilization, pour agar medium (5.3.4) into sterile Petri dishes to give a depth of about 5 mm. It solidifies on cooling.

7.2.3 Test for without solid-medium (optional)

Sterile Petri dishes without medium are used for this part. This is important in the case of the sanitary sealant where the necessary scale is 0. The test without medium is to eliminate potential artefact of growth that can occur with the test on medium.

7.2.4 Arrangement of test specimens

Place the specimens (5 without immersion and 5 after immersion) separately, as flat as possible, on the solidified medium, avoiding any contact between specimens or with the walls of the Petri dishes. In the case of without solidified medium (5 without immersion and 5 after immersion), place the specimens on the Petri dishes as flat as possible.

7.2.5 Preparation of spore suspension

Produce a spore suspension from well sporulated cultures, using mineral-salt/wetting-agent solution (5.3.2).

7.2.6 Harvesting the spores

Apply 5 ml of mineral-salt/wetting-agent solution (5.3.2) to a well sporulated agar plate. Gently scrape the surface of the culture with a sterile inoculation needle to obtain an aqueous suspension of the spores. Harvest the spore suspension by pipetting it into a fresh test tube. Repeat this procedure with the same culture three times. Then shake the spore suspension of each fungal culture with sterile glass beads and filter through a thin layer of sterile cotton or glass wool to remove mycelial fragments.

7.2.7 Washing the spores by centrifugation and preparation of working suspensions

Aseptically centrifuge the filtered spore suspension and discard the supernatant liquid. Re-suspend the residue in 25 ml of stock mineral-salt solution (5.3.1) and centrifuge again. Suspend the washed residue in 50 ml of stock mineral-salt solution. This repeated washing of the spore suspensions is intended

to guarantee that all surface-active substances are removed which might cause stress cracking in polymers.

Adjust the concentration to about 10^6 spores per millilitre (determined using a counting chamber or by turbimetry). The final spore solution should be done in mineral-salt/glucose solution (5.3.3).

Repeat these operations with each test fungus. Blend equal volumes of five suspensions containing the same number of spores to obtain the final mixed spore suspension ready for inoculation. Use the spore suspension within 2 days of-preparation (the spore suspension should be placed between 3 °C to 10 °C if it is not used within 6 h).

7.2.8 Spore viability check

Fill two sterile Petri dishes with agar medium (5.3.4), following the procedure given in 7.2.2 and inoculate with one drop of each of the spore suspensions (before blending the spore suspension). Incubate at (29 ± 1) °C for 3 to 4 days (carry out the viability check at the same temperature as the actual determination). In the absence of copious growth, prepare a new spore suspension from fresh cultures and repeat the test.

7.2.9 Inoculation of specimens

Put the specimen on the agar, then spray 0,1 ml spore suspension on the surface of specimens and agar.

NOTE Usually it's hard to smear spore suspension evenly on the surface of silicone sealant because of the hydrophobicity.

7.2.10 Incubation

Incubate the inoculated 10 or 20 specimens at (29 ± 1) °C and at a humidity > 90 % for 4 weeks, or longer by agreement between the interested parties. Take precautions to prevent condensed water dropping onto the surface of the specimens. If the test lasts more than 4 weeks, re-inoculate the specimens every 4 weeks using washed and centrifuged spores.

The fungal growth is evaluated (8.1) after 4 weeks incubation.

In case of extended incubation periods, the specimens are re-inoculated with the spore suspension (7.2.5) in 4-weeks intervals.

8 Assessments

8.1 Assessment of fungal growth on the specimens by visual examination

First examine the exposed specimens with the naked eye and then, if necessary, with a stereoscopic microscope (at a magnification of × 50). Assess the fungal growth in accordance with the scale given in Table 3.

If the results of the visual examination of the specimens vary by more than two scale ratings, repeat the determination with fresh specimens.

The examination of the cleaned specimens can supply further information, Colour photography is a useful aid in recording the results of the visual examination.

Table 3 — Assessment of fungal growth

Grade (Intensity of growth)	Evaluation
0	No growth apparent under the microscope.

Table 3 *(continued)*

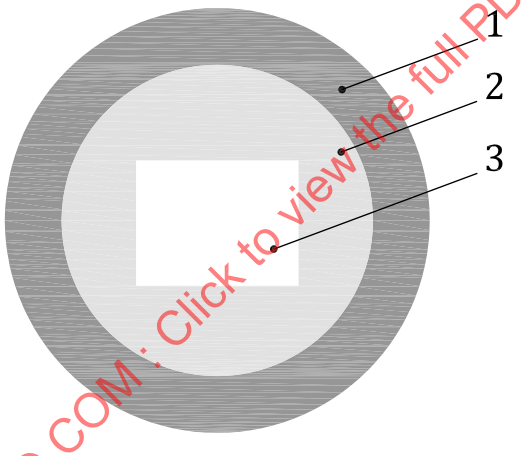
Grade (Intensity of growth)	Evaluation
1	No growth visible to the naked eye, but clearly visible under the microscope.
2	Growth visible to the naked eye, covering up to 25 % of the test surface.
3	Growth visible to the naked eye, up to 50 % of the test surface.
4	Considerable growth, covering more than 50 % of the test surface.
5	Heavy growth, covering the entire test surface.

It is suggested that a grid be placed over the specimen to enable percentage surface growth to be assessed (see ISO 846:2019, Annex C).

8.2 Assessment of inhibition zone

Some types of test specimens have inhibition zone after the test.

Figure 3 is the definition of the inhibition zone. The inhibition zone is usually the space between the sealant area and the fungi formation zone. The purpose of the measurement of inhibition zone is to identify the characteristic of the leaching of the sealant.



Key

1 fungi formation zone
2 inhibition zone
3 sealant specimen

Figure 3 — Definition of inhibition zone

For the evaluation of the inhibition zone size, see Figure 4.