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Byggnadsmaterial – Laboratoriemetod inför bedömning av lägsta hygrotermiska förhållanden för mögelpåväxt

Building materials – Laboratory method for assessment of the lowest hygrothermal conditions required for mould growth

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Innehåll

Introduction

Building materials that are stored or used in damp environments may be subject to mould growth. The susceptibility of building materials to mould growth varies. Some materials withstand high relative humidity without mould growth occurring, while on others mould can grow at as low as 75 % relative humidity. In a building, different construction parts are exposed to various humidity and temperature conditions. To minimize the risk of microbial growth, materials should be chosen and handled in a way so they can tolerate the conditions in question.

This Technical Specification describes a method for determining the critical moisture level of mould growth at one specific temperature on the surface of a building material after 12 weeks of humid exposure. The results may be used to theoretically estimate the critical moisture value at other temperatures. The tests are performed during constant incubation conditions at one set temperature and four levels of relative humidity.

When designing this method a number of standardized methods and regulations were taken into consideration [1], [2], [3], [4], [5], [6], [7] together with data and practical experience from several Swedish research projects [8], [9] and [10]. The method provides conditions for good repeatability by the requirements which describe technical equipment, incubation and material handling.

1 Scope

This Technical Specification describes a laboratory test method for assessing the critical moisture levels at one specific temperature after 12 weeks of humid exposure regarding mould growth on building materials. The results can be used to theoretically estimate critical moisture level at any other temperatures than the tested. The method is applicable for clean materials that have not previously been exposed to conditions that will enable mould growth.

The method

— is not applicable for prediction of mould growth beyond the time tested in the laboratory;

— do not consider health effects of mould exposure in the indoor environment;

— cannot be applied to outdoor building structures, e.g. facades, fences etc., where rain, wind, radiation from the sun etc. may affect mould growth;

— do not include growth of e.g. bacteria, although this also contribute to the microbial fouling of materials in damp buildings.

2 Normative references

The following referenced documents are indispensable for the application 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.

EA-4/02 M: 1999, *Expression of the Uncertainty of Measurement in Calibration. Guidance document to ISO/IEC 17025 and ISO 15189. Application and Technical/Advisory documents,* European co-operation for Accreditation, www.european-accreditation.org

SS-ISO 3696:1987, *Water for analytical laboratory use – Specification and test methods*

3 Terms and definitions

For the purposes of this Technical Specification, the following terms and definitions apply:

3.1
expanded uncertainty
quantity defining an interval about the results of measurements that can be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurands

Note 1 to entry: In this technical specification the measurands are temperature and relative humidity.

[SOURCE: ISO 3534-2:2006]

3.2
relative humidity
RH
measure of the amount of water vapour in the air (at a specific temperature) compared to the maximum amount of water vapour air could hold at that temperature and is given as a percentage value

3.3
critical moisture level
$RH_{crit\ T}$
relative humidity (%) at which mould growth appears at a certain temperature T(°C)

Note 1 to entry: The abbreviated form normally includes the temperature value, for example $RH_{crit,\ 22\ °C}$.

4 Principle

Specimens of the material to be tested shall be inoculated with a suspension of a mixture of spores from each of six species of mould fungi and shall then be incubated in test chambers at specified temperature and relative humidity conditions during 12 weeks. At defined time intervals, the specimens shall be visually inspected for fungal growth at a magnification of 40x. The fungal growth shall be assessed according to a rating scale. The critical moisture level for mould growth at a certain temperature shall be determined by defining a range with minimum relative humidity at which established growth of mould is detected during the test period.

5 Apparatus

5.1 General

All apparatus shall be serviced and calibrated at regular intervals.

5.2 Test chambers

Incubation chambers that can provide the required conditions shall be used. They shall maintain temperature and humidity with good homogeneity in all parts of the chambers. The fluctuations shall not be larger than $\pm$ 2 °C and ± 2,5 % RH during operation. The required conditions shall be restored within two hours after opening and closing of the chamber.

A test chamber with salt solutions shall not be used as it may affect the mould growth, since the salt can migrate.

NOTE Lighting in the chambers is not required.

5.3 Device for recording incubation conditions

Each test chamber shall be equipped with a separate transmitter with a data logger for recording temperature and relative humidity.

The transmitter shall be calibrated at least once a year if continuously used. Otherwise it shall be calibrated prior to the time of use.

The expanded uncertainty of measurement of the relative humidity and temperature shall be considered according to 10.8 and Annex B.

5.4 Inoculation device

An airbrush connected to a compressor, or to small cans with compressed air, shall be used to spray the spore suspension onto the surface of the specimens to be tested. A filter or regulator that removes moisture from the compressed air should be connected to the compressor.

NOTE The most effective way is to use an airbrush where the cup for the liquid is open and placed directly on the air-brush.

5.5 Stereo microscope

A stereo microscope with magnification of 40x shall be used in all analyses. An external, adjustable light source shall be used to illuminate the whole surface of the specimen at a low angle.

5.6 Laminar air flow bench

A laminar air flow bench shall be used to provide biological safety throughout the process of making the spore suspension.

NOTE A continuous unidirectional horizontal air flow prevents contamination from operator and the environment.

5.7 Autoclave

An autoclave shall be used to sterilize reagents, materials and other utensils for the test.

5.8 Centrifuge

A centrifuge shall be used for separating spores from the water solution when cleaning the spores in the process of preparing the spore suspension.

6 Reagents and laboratory material

6.1 Purity of water

The water used shall be according to the classification grade 3 in SS-ISO 3696:1987.

6.2 Nutrient media

6.2.1 Malt extract agar

A minimum of 25 Petri dishes shall be prepared for cultivation of fungi and for viability controls. The culture medium shall be a malt agar medium with the following recipe:

20 g Agar agar (purified and free of inhibitors) mixed with 20 g malt extract to 1000 ml water and autoclaved at 121 °C for 15 minutes.

6.2.2 Malt solution

A 250 ml malt solution shall be prepared for incubation environment control with the following recipe:

20 g malt extract per 1000 ml water, autoclaved at 121 °C for 15 minutes.

6.3 Test organisms

6.3.1 Test fungi

The test fungi in Table 1 shall be used.

Table 1 – Test fungi

Test fungus	CBS number
Eurotium herbariorum	115808
Aspergillus versicolor	117286
Penicillium chrysogenum	401.92
Aureobasidium pullulans	101160
Cladosporium sphaerospermum	122.63
Stachybotrys chartarum	109292

NOTE CBS number refers to strains maintained by Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

6.3.2 Maintenance of test fungi

Subsequent inoculation from the original prepared cultivation of test fungi on new Petri dishes (see 10.2) shall be repeated no more than 12 consecutive months in order to avoid degeneration of the fungi. Each Petri dish with prepared stock culture shall not be kept for more than 4 months at 8 ± 2 °C before it is used in a test.

6.4 Laboratory material

6.4.1 Culture vessels

Petri dishes shall be used as culture vessels. They shall have a diameter about 90 mm and a height of about 15 mm.

6.4.2 Flasks

Six glass flasks, each with a volume of 250 ml, shall be used for the spore solution production process.

6.4.3 Glass beads

About 100 cl of glass beads, about 5 mm in diameter, shall be used in the spore solution production process.

6.4.4 Control glass microfiber filters

Glass fibre filters, binder-free and not containing any cellulose, 55 mm in diameter shall be used for controls.

6.4.5 Glass funnels

Six glass funnels shall be used in the spore solution production process.

6.4.6 Washed glass wool

Washed glass wool for laboratory use shall be used as a filter in the glass funnels.

6.4.7 Counting chamber

A counting chamber or equivalent equipment shall be used when calculating the concentration of spores in the suspension. Handling and calculation shall be done according to the manufacturer’s instructions.

6.4.8 Burner

A burner or a spirit lamp shall be used to sterilize the preparation needle during the process of preparing the spores suspension.

6.4.9 Additional supplies for production of spore solution

Additional supplies that shall be used for the production of spore solution are: Automatic pipettes, pipette tips, forceps, inoculation needle or spreader, centrifuge tubes, Petri dishes, stainless steel mesh, latex or plastic gloves, face masks.

7 Test specimens

7.1 General

To minimize organic contamination, all handling of the material to be tested shall be carried out by using clean gloves and clean tools.

7.2 Preparation of test specimens

The material selected for the test should be representative, clean and previously unused material. Preferably, the material should originate from different batches in the production. This is particularly important if the quality is expected to vary between batches.

Prepare seven specimens of the material for each of the four incubation sets. Each specimen should be about 50 mm x 100 mm. If the two surfaces of the test material have had different treatments or consists of different materials or structures, duplicate specimen sets are required as each side should be tested separately.

When testing *in situ* formed loose-fill thermal insulation or similar materials, each material sample shall be placed in an autoclaved cage of fine stainless steel mesh with an internal volume of approximately 50 ml. The density in the cage should be according to the stipulated density for the integrated product or the density recommended in any applied standard.

NOTE The method does not require pre-conditioning of the test specimens. It has been validated in separate experiments for different building materials that the initial moisture content of test specimens has no impact on the critical moisture level assessed after 12 weeks [8] when using test chambers with the specified properties in section 5.

7.3 Reference specimens

When evaluating the efficiency of a treatment, seven untreated specimens of the material for each of the four incubation sets shall be prepared from the same material and used as references.

NOTE A treatment such as a detergent, a coating or added fungicide.

8 Procedure

8.1 General

To ensure aseptic conditions during the preparation of spore suspension and inoculation of test specimens, all equipment shall be sterilized before use. Perform the work with clean gloves in a laminar air flow bench. The relevant procedures for good biological practise in [7] should be applied.

8.2 Cultivation of test fungi

Prepare strains according to instructions from the supplier. Cultivate each species on separate Petri dishes with malt agar until heavy sporulation has been developed. Usually this will occur within four weeks. Prepare a minimum three Petri dishes for each strain. Previously prepared cultures may be used according to 6.3.2.

8.3 Preparation of spore suspension

The spore suspension shall be inoculated on the test specimens immediately after the production or within 12 hours after preparation. The following steps shall be performed in successive order:

a) Prepare six flasks with 35 ml distilled water and a layer of glass beads and autoclave at 121 °C for 15 minutes. Allow to cool.

b) Put a thin layer of glass wool in six glass funnels and autoclave at 121 °C for 15 minutes. Allow to cool.

c) Verify the purity of each culture, i.e. that each Petri dish contains the correct mono-culture.

d) Pour 10 ml of distilled, autoclaved water onto each of the Petri dishes with one of the subcultures and scrape the surface of the fungal colonies gently and carefully with a clean inoculation spreader or inoculation needle. Repeat for each subculture.

e) Pour the liquids into the prepared sterile flasks containing glass beads and autoclaved water, one for each species. Shake the flasks vigorously to liberate the spores from conidiophores and to break up any large spore clumps.

f) Filter the liquid through the sterile glass wool in the glass funnel into a centrifuge tube, one for each species.

g) Centrifuge the tubes with suspension until a spore pellet is formed.

h) Pour off the supernatant and fill the tubes with new water and centrifuge in the same manner as before. Repeat this washing procedure three times after the first centrifugation, so that any nutrients from the agar that could affect the test results are washed out and hyphae are avoided in the final solution.

i) Determine the spore concentration in the final washed residue re-suspended in about 20 ml sterile de-ionized water for each species using a counting chamber under the microscope. Adjust the spore concentration with sterile deionized water to approximately 1 x 106 spores per ml.

j) Prepare the final spore suspension by mixing equal volumes of suspension from each species.

8.4 Inoculation

8.4.1 Inoculation of test specimens

Spray 0,4 ml of the spore suspension onto one surface of each test specimen by using an airbrush (see 5.3). During spraying, sweep the airbrush along at an even speed so the spores become evenly distributed over the surface.

8.4.2 Inoculation of positive control #1 – Viability of spore suspension

In order to check the viability of the spore suspension and each species of fungi, inoculate separate Petri dishes (see 6.2.1) with one or two droplets of each of the spore suspensions from the individual species. Spray 0,4 ml of the mixed spore suspension onto one Petri dish with malt agar (see 6.2.1). Incubate the Petri dishes, with lid on, in room temperature or higher, maximum 30 °C.

8.4.3 Inoculation of positive control #2 – Test chamber environment

In order to check the test chamber environment in each cabinet, dip four glass microfiber filters (see 6.4.4) into malt solution (see 6.2.2) and allow them to dry. Then spray 0,2 ml of the spore suspension onto each filter paper and place in separate, empty, Petri dishes, with the lid off. Incubate one filter together with inoculated test specimens in each test chamber.

NOTE A second positive control (without lid) may be placed in each test chamber to check possible influence to the incubation environments from materials treated with volatile inhibitors (see 9.2). The double positive controls should have equal growth rate.

8.4.4 Inoculation of negative control – Nutrient purity of spore suspension

In order to check the nutrient purity of the spore suspension, inoculate one sterile microfiber filter (see 6.4.4) by spraying with 0,2 ml of the spore suspension. Place it in an empty sterile Petri dish and incubate in the test chamber, with the lid off, at 95 % RH and 22 °C.

8.5 Incubation

The test shall be performed at 22 °C and for in total 12 weeks at four levels of relative humidity: 80 % RH, 85 % RH, 90 % RH and 95 % RH. The temperature and relative humidity shall be recorded every 10 minutes during the test period. Evaluate the incubation conditions throughout the test according to 8.7.

The test shall be performed in one of the following ways: by starting all four tests at different levels of humidity using the same spore solution to all specimens and separate incubation cabinets or by starting each case of RH condition successively in the same incubation cabinet. Consequently in the latter case: each start shall