Chapter 5
Analytical and Experimental Methods for the Assessment of the Biological Proliferation in Buildings

Abstract In order to preserve buildings from the colonisation of microorganisms and to act efficiently against biodeterioration, it is necessary to have a better understanding of biodeterioration mechanisms and their effects on materials properties. Consequently, there is a growing demand for calculation methods in building engineering to assess the moisture behaviour of building components and microorganism risk prediction in order to ensure a healthy environment and to avoid defacement of materials and other social and economic consequences. Many building hygrothermal analysis methods are able to simulate the coupled transport processes of heat and moisture for one or multidimensional cases, aiming to predict biological risk. Additional measurements in laboratory and in situ conditions have been used for the validation of these models. In the first part of this chapter, we review some of the major biological risk predictive models, both inside and outside the buildings. Then, in the second part, we will describe some methods of accelerated experimental testing for the evaluation of biological defacement of building materials. A more in-depth study of microorganism growth under transient conditions is still necessary in order to define the most reliable prediction model. To do so, additional measurements in laboratory and in situ conditions on new Nearly Zero Energy Buildings and components would be desirable.

Keywords Condensation · Time of wetness · VTT model · Isopleth · Biohygrothermal model · Accelerated test · Biodeterioration

5.1 Analytical Models

In literature there are many hygrothermal analysis methods to simulate the coupled transport processes of heat and moisture for one or multidimensional cases.

Hundreds of building software tools based on these methods have been developed or enhanced to be used for the prediction of the hygrothermal performance of buildings. These models vary significantly concerning their

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mathematical sophistication that depends on the degree that takes into consideration the following parameters: moisture transfer dimension; type of flow (steady-state, quasi-static, or dynamic); quality and availability of information and stochastic nature of each data (material properties, weather, construction quality, etc.) (Delgado et al. 2010).

As the purpose of most hygrothermal models is usually to provide sufficient and appropriate information needed for decision-making, the software should be available in the public domain (freeware or commercially) and it should be "user friendly" (Delgado et al. 2010).

In the following sections, we will see some of the most known models for the evaluation of the development of microorganisms on the facades and inside of buildings.

5.1.1 Models of Biological Growth on Facades

Many studies have pointed out that microbiological growth on building facades is due to the high values of surface moisture content, which results from the combined effect of four parameters: surface condensation, wind-driven rain (WDR), drying process and properties of the exterior layer.

Most analytical methods of the envelope hygrothermal performance are developed in this way: they are useful tools in assessing exterior condensation on façades and the importance of radiative balance on the exterior surface temperature. The periods of surface condensation and the accumulated degree of cooling below dew point temperature are taken as criterion to classify the resulting biological growth (Krus et al. 2006).

However, no simple method has yet been developed to predict the risk of the biological defacement of the façade similarly to what has already been done for mould growing on interior finishes, where the mould spore hygrothermal behaviour is taken into account.

Surface humidity is considered as the principal criterion for assessing the risk of biological growth. Nevertheless, the comparison of simulated values with the results of ''in situ'' tests performed on a façade covered with ETICS showed that there is no good agreement between the simulated and the measured values of the relative humidity, especially when wind-driven rain is taken into account (Delgado et al. 2010).

Recent studies have started to develop a simple process to predict the risk of biological defacement of building facades, also by analysing experimental data on growth.

An interesting attempt is the model "BIO.MOD", which defines three risk indices (Eqs. 5.1, 5.2 and 5.3) that are related to the surface humidification, by

condensation (CPE_a), due to WDR (WDRPEa) or due to the sum of the two, with the maximum drying capacity (DPE_a) (Barreira and Freitas 2011):

$$\text{BIO.MOD1} = \frac{\text{CPE}_a}{\text{DPE}_a} * 10^3 \left[\frac{\text{Pa} * \text{h}}{\text{Pa} * \text{h}}\right] \tag{5.1}$$

$$\text{BIO.MOD2} = \frac{\text{WDRPE}_a}{\text{DPE}_a} * 10^3 \left[\frac{\text{kg/m}^2}{\text{Pa} * \text{h}}\right] \tag{5.2}$$

$$\text{BIO.MOD3} = \frac{\text{CPE}_a + \text{WDRPE}_a}{\text{DPE}_a} * 10^3 \left[\frac{\text{Pa} * \text{h} + \text{kg/m}^2}{\text{Pa} * \text{h}}\right] \tag{5.3}$$

For Barreira and Freitas, exterior surface condensation can be analysed using psychrometry principles (Barreira and Freitas 2011). When water vapour partial pressure of the air is greater than the water vapour saturation pressure on the surface, condensation will occur (Hagentoft 2001). According to Zheng et al., the difference between the water vapour partial pressure in the air (Pv(air), in Pa) and the water vapour saturation pressure on the surface (Psat(surface), in Pa) may be called Condensation Potential (CP, in Pa), which implies condensation for positive values (Zheng et al. 2004). CP can be understood as the amount of water vapour that is available to condensate (Eq. 5.4).

$$\text{CP} = \text{P}_\text{v}(\text{air}) - \text{P}_\text{sat}(\text{surface}) \tag{5.4}$$

To evaluate the amount of condensation, positive CP and its lasted time should be considered. The product of positive CP (CP (>0), in Pa) by its lasted time (Δt_{CP} (>0), in h) may be called Condensation Potential Equivalent (CPE, in Pa) and allows the estimation of the risk of condensation for a certain period of time (Eq. 5.5). In order to estimate the risk of condensation for a certain period of time CPE must be accumulated in time (CPEa).

$$\text{CPE} = \text{CP}_{(>0)} * \Delta t_{\text{CP}_{(>0)}} \tag{5.5}$$

The humidification of a façade due to wind-driven rain (WDR) may be assessed, for a certain period of time, through the WDR Potential Equivalent (WDRPEa, in kg/m^2), which is obtained by integrating the intensity of WDR (in kg/(m^{2}s) in time. WDRPEa has to be multiplied by 100 in order to reach values that are comparable with CPEa values (Eq. 5.6).

$$\text{WDRPE}_a = 100 * \int_0^t \text{WDRdt} \tag{5.6}$$

Similar to condensation, also the drying capacity of a wet surface can be analysed using psychrometry principles (Hagentoft 2001). By analogy, it is possible to establish the concept of Drying Potential (DP, in Pa), as being the difference between the water vapour saturation pressure on the surface (Psat(surface), in Pa) and the water vapour partial pressure in the air (Pv(air), in Pa), which

implies evaporation for positive values (Eq. 5.7). DP can be understood as the amount of water vapour transferred to the air, considering that the surface remains permanently wet.

$$\mathrm{DP} = \mathrm{P_{sat}(surface)} - \mathrm{P_v(air)} \tag{5.7}$$

In order to evaluate the maximum ability to dry out, the product of positive DP (DP(>0), in Pa) by its lasted time (ΔtDP(>0), in h) shall be considered and may be called Drying Potential Equivalent (DPE, in Pa) (Eq. 5.8). In order to estimate this ability for a certain period, DPE must be accumulated in time (DPEa).

$$\mathrm{DPE} = \mathrm{DP}_{(>0)} * \Delta t_{\mathrm{DP}_{(>0)}} \tag{5.8}$$

It must be stated that DPEa is not useful as a parameter for modelling the real drying capacity of a wet surface, as this is not permanently saturated. After some time, the liquid water evaporates and the vapour pressure at the surface depends not only on the surface temperature, but also on its relative humidity. However, in order to avoid the use of relative humidity and to simplify the parameters used in the drying process assessment, DPEa can be employed as an overvalued drying capacity.

Using the data collected during an "in situ" campaign, Barreira and Freitas calculated in annual bases CPEa, WDRPEa, DPEa and the three indices of BIO.MOD (Barreira and Freitas 2011). They found a good agreement between the index BIO.MOD3, which combines surface condensation with the effect of WDR and "quantifies" the risk of defacement due to biological growth, and the accumulated hours of surface saturation (relative humidity equal to 100 %), measured simultaneously. Using the model BIO.MOD they also affirmed that the drying process is the most relevant parameter and that surface condensation has more impact than WDR. The authors finally establish a risk map for walls covered with ETICS located in the Portuguese territory.

The development of further models, which take into account the types of microorganisms proliferating, still requires more research and analysis of experimental data.

5.1.2 Models of Internal Mould Growth

In order to better understand the phenomena of formation of mould on the construction elements, dynamic models are needed, which are able to consider not only the variations of the internal and external boundary conditions of relative humidity and temperature, but also the time required for the growth of mould. These are the cardinal influencing factors in the mould growth process (Adan 1994). The critical value for these parameters can however differ for each mould species.

While in the past the temperature ratio was often used to minimise the risk of mould, nowadays more advanced mould prediction models can be found. These models include the main influencing factors for mould growth, which are surface temperature and relative humidity.

Next sections will give an overview of the different existing models on the mould risk evaluation.

5.1.2.1 IEA-Annex 14

IEA-Annex 14 (IEA 1991; IEA 1990) stated that:

- Surface condensation starts each time the relative humidity (RH) on a surface reaches 100 %, that means, each time the vapour pressure (P) in the air against the surface equals or becomes higher than the saturation pressure on the surface (P'si):
  Surface condensation when $P \geq P'si$
- Mould germination becomes possible when the mean water activity against/on a nutrient surface remains higher than a threshold value 'a', 'a' being a function of the mould species, the temperature, the substrate (nutrient), etc.… Using the fact that, in steady-state, the water activity is nothing other than the RH, the mould condition becomes:
  Mould germination when $P \geq a*P'_{si}$

IEA-Annex then defined a surface relative humidity threshold for mould growth dependent on the elapsed time, based on the lowest isopleth for *Aspergillus versicolor*: 80, 89 or 100 % for an exposure time of 1 month, 1 week and 1 day, respectively.

The Annex also defined a design value for the temperature ratio (Eq. 5.9):

$$\tau = \frac{\theta_{s,\min} - \theta_e}{\theta_i - \theta_e} \geq 0.7 \tag{5.9}$$

With $\theta_{s,\min}$(°C) being the minimum indoor surface temperature and θ_i and θ_e the inside and outside temperature (°C), respectively. A temperature ratio of 0.7 is proposed as criterion, related to an acceptable mould risk of 5 %. A lower ratio introduces an unacceptable high mould risk.

Although the temperature ratio is often used as a design criterion, an in situ study on 35 mould infested dwellings indicated that this criterion may not be used as a stand-alone performance because of the importance of other factors such as low ventilation rate, rain infiltration, less heating or thermal bridges (Hens 2003).

Furthermore, a relative humidity threshold of 100 % in cases of 1-day exposure is questionable since liquid water hinders mould development. A threshold of 99 % relative humidity is more plausible (Vereecken and Roels 2012).

5.1.2.2 Model of Time of Wetness

Adan studied fungal growth not only under steady-state but also under transient indoor conditions, in order to improve the understanding of the process which induces the fungal defacement of interior finishes (Adan 1994). He particularly investigated the response of the fungal cell to transient water vapour pressures, and observed that fungi are capable of an instantaneous water vapour uptake as the RH increases, suggesting that short periods of high RH should not be neglected in the evaluation of indoor climate with respect to mould growth.

In order to indicate the water availability under transient conditions, he introduced the time of wetness (TOW), defined by the ratio of the cyclic wet period (i.e. when the RH $\geq$ 80 %) and the cyclic period (Eq. 5.10):

$$\mathrm{TOW} = \frac{\text{cyclic wet period } (\mathrm{RH} \geq 80\%)}{\text{cyclic period } (\text{wet} + \text{dry})} \tag{5.10}$$

His preliminary experiments indicated that the growth of *Penicillium chrysogenum* on gypsum-based finishes is only weakly affected for a TOW $\leq$0.5. The RH value during the drying periods hardly shows any influences on the fungal growth-TOW relation. Furthermore, except for very fast oscillations of the RH, the frequency of high RH periods only slightly affects the TOW effects on fungal growth.

Besides, Adan developed sigmoid curves, which enable a satisfactory fit of the mould growth rate of *P. chrysogenum* on gypsum board material. In this, the average rating is defined as in the BS3900 standard (Table 5.1).

By the way, Adan's experiments were limited to measured data on gypsum board materials inoculated with *P. chrysogenum*, so his model cannot be used to predict fungal growth in cases of other substrates or species.

5.1.2.3 VTT Model

The VTT model is an empirical mould prediction model developed by Hukka and Viitanen (1999), in which quantification of mould growth is based on the mould index (M) used in the experiments on wooden materials for visual inspection (already seen in Table 4.1). The index can be used as a design criterion, e.g. often

Table 5.1 Rating scale according to mould coverage area in the model of Adan (1994)

Rating	Coverage area
0	No mould growth
1	Coverage $\leq$1 %
2	1 %$\leq$ Coverage $\leq$10 %
3	10 %$\leq$ Coverage $\leq$30 %
4	30 %$\leq$ Coverage $\leq$70 %
5	70 %$\leq$ Coverage

a mould index equal to 1 is defined as the maximum tolerable value since from that moment the germination process is assumed to start.

With the exception of the maximum index of 6, the definition of the mould indices agrees very well with the definition for the rating used by Adan (Table 5.1).

The model consists of differential equations describing the growth rate of the mould index in different fluctuating conditions, including the effect of exposure time, temperature, relative humidity and dry periods.

The incremental mould index can be calculated by using a differential equation.

For pine and spruce, the incremental change in mould index (M) is given by Eq. 5.11:

$$\frac{dM}{dt} = \frac{1}{7\exp(-0.68\ln T - 13.9\ln RH + 0.14W - 0.33SQ + 66.02)} k_1 k_2 \quad (5.11)$$

where T (0,1–40 °C) is the ambient temperature, RH (%) is the Relative Humidity, W the wood specie (0 for pine, 1 for spruce), SQ the surface quality (0 for resawn and 1 for original kiln-dried timber), the factor k_1 defines the growth rate under favourable conditions, and k_2 represents the response time for the beginning of mould growth.

The main disadvantage of the first VTT model is its limitation to unpolluted spruce and pine softwood. This type of wood is widely used in Scandinavia, but hardly used for building construction elsewhere.

Recently, the VTT model was expanded for other building materials (Ojanen et al. 2010): spruce board (with glued edges), concrete (K30, maximum grain size 8 mm), aerated concrete, cellular concrete, polyurethane thermal insulation (PUR, with paper surface and with polished surface), glass wool, polyester wool and expanded polystyrene (EPS). Pine sapwood was used as a reference material.

The mould growth parameter values of different materials were adapted to the existing model. Some improvements were applied for the model structure to better adjust different growth phenomena.

The main difference with the original model was found at microscopic level, since for some materials already at this level a rather high mould growth coverage could be observed. Therefore, the mould index determination was updated with these microscopic growth coverage findings in index levels three and four.

The principle when updating the original mould growth model for other materials was that new values for the factors presented in Eq. 5.11 were determined for these materials using the results from several experiments. To improve the usability of these new values, they were not presented as exact values for each material but as “material classes” according to the sensitivity of the material to mould growth. Four different sensitivity classes were determined (Table 5.2).

The presented improvements of the mould growth simulation model do not guarantee the exact prediction of mould in all cases and conditions. The new model values were determined based on a limited set of experiments with relatively large scattering and should therefore be interpreted as a first approximation.

Table 5.2 Mould sensitivity classes (Vereecken and Roels 2012)

Sensitivity class	Material in experiment	Material groups
Very sensitive	Pine sapwood	Untreated wood; includes lots of nutrients for biological growth
Sensitive	Glued wooden boards, PUR with paper surface, spruce	Planed wood; paper-coated products, wood-based boards
Medium resistant	Concrete, aerated and cellular concrete, glass wool, polyester wool	Cement or plastic based materials, mineral fibres
Resistant	PUR with polished surface	Glass and metal products, materials with effective protective compound treatments

The variation of the material sensitivities is high, estimation of a product sensitivity class is difficult without testing, the surface treatments may enhance or reduce growth potential, different mould species have different requirements for growth, and the evaluation of the actual conditions in the critical material layers may include uncertainties.

5.1.2.4 Isopleth Models

Isopleth curves represent the relation between the mould risk and the main mould inducing factors (relative humidity or water activity, temperature, exposure time). These curves separate favourable from unfavourable conditions for mould growth. The simplest models just provide the limit state curve, more advanced isopleth models subdivide in time until germination and growth rate.

In the model developed by Clarke et al. the mould fungi found in buildings are subdivided into six categories, ranging from xerophilic (dry loving) to hydrophilic (wet loving) fungi (Clarke et al. 1997, 1999). Each category constitutes a family of mould species possessing similar growth requirements over the range of temperature and humidity conditions likely to be found in the indoor environment. Each category is accompanied with a representative fungus:

- Highly xerophilic: *Aspergillus repens*
- Xerophilic: *A. versicolor*
- Moderately xerophilic: *P. chrysogenum*
- Moderately hydrophilic: *Cladosporium sphaerospermum*
- Hydrophilic: *Ulocladium consortiale*
- High hydrophilic: *Stachybotrys atra*.

For each of these categories a growth limit curve defined by a third-order polynomial function was determined based on an analysis of published data. When the relative humidity and temperature combination exceeds such a curve, mould growth of the matching fungi will occur (Fig. 5.1).

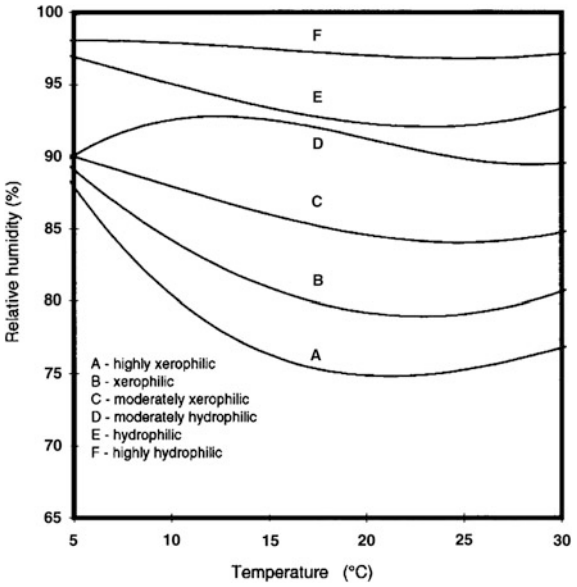

Fig. 5.1 Limiting growth curves for six mould growth categories. Reprinted from Clarke et al. (1999), copyright 1999, with permission from Elsevier

The mould growth prediction model was developed in the ESP-r modelling system for the assessment of the environmental and energy performance of buildings. This model is able to predict the time series evolution of the local surface temperature and relative humidity while taking explicit account of constructional moisture flow and local air movement. The mould growth limit curves are contained in a moulds database. This allows the predicted local conditions to be plotted directly to the mould growth curves. The concentration of plotted points relative to the various growth bands allows an assessment to be made of the risk of mould growth and its persistence over time.

Because the mould growth curves were generated from experiments in which the principal determinants of growth were maintained at fixed values, the model is unable to indicate the effects on mould growth of temperature and/or humidity fluctuations over prolonged periods of time.

Since in the model the exposure time is not taken into account, a single excess of the isopleths is set equal to mould formation, consequently this model assumes the worst-case scenario and risks to overestimate the mould risk on finishing materials.

Among the moulds frequently found on defaced finishes in buildings, the xerophilic species *A. versicolor* has the isopleth with the lowest relative humidity for germination (IEA 1991). This lowest isopleth is closely matched by the following parabolic relation (the relative humidity RH in %, temperature θ in °C) (Hens 1999) (Eq. 5.12):

$$\mathrm{RH}_{\mathrm{threshold}} = 0.033\theta^2 - 1.5\theta + 96 \tag{5.12}$$

Giving 79 % at 22.7 °C. At 10 °C, the threshold raises to 84 %. For shorter periods, a logarithmic function is suggested (Eq. 5.13):

$$RH_{threshold} = \min\{1, 0.8[1.25 - 0.075\ln(t)]\} \quad (5.13)$$

where t is the time (days). This function developed for shorter periods does not take into account the influence of the temperature.

Because of the abundance of mould species and materials, an individual isopleth system for each species and substrate is not possible. Therefore, in order to expand the existent isopleth system to date, Sedlbauer subdivided the mould species and materials found in buildings in a set of classes (Sedlbauer 2002). A first subdivision was based on the health risk of the different mould species:

- Class *A*: mould species which are highly pathogen and consequently not allowed to occur in buildings;
- Class *B*: mould species which are pathogen when exposed over a longer period or which cause allergic reactions;
- Class *C*: mould species which are not dangerous to health.

Based on measurements for a collection of species, minimum growth conditions for the three hazardous classes were obtained. For classes *B* and *C* only slightly different results were obtained, so that classes *B* and *C* were combined in one class *B*/*C*.

The conditions below where no spore germination or growth will occur was indicated by the LIM (Lowest Isopleth for Mould)-curve. This was developed based on the lowest envelope of all the lowest curves of the group.

After the determination of the LIM-curves, representative mould fungi for the different hazardous classes were searched. They had a LIM-curve which approximated the LIM-curve described above. For class A this representative fungus is *A. versicolor*, for class B/C the mould fungi are *Aspergillus amstelodami, Aspergillus candidus, Aspergillus ruber* and *Wallemia sebi.* Based on the growth of these representative fungi on an optimal culture medium, the isopleth systems for the hazardous classes were developed.

The influence of building substrate, i.e., the building material itself and its possible soiling, was then taken into account by a second subdivision in the following categories (Sedlbauer 2002):

Substrate category 0—Optimal culture medium;

Substrate category I—Biologically recyclable building materials like wall paper, plaster cardboard, building materials made of biologically degradable raw materials, material for permanent elastic joints;

Substrate category II—Biologically adverse recyclable building materials such as renderings, mineral building material, certain wood as well as insulation material not covered by I;

Substrate category III—Building materials that are neither degradable nor contains nutrients.

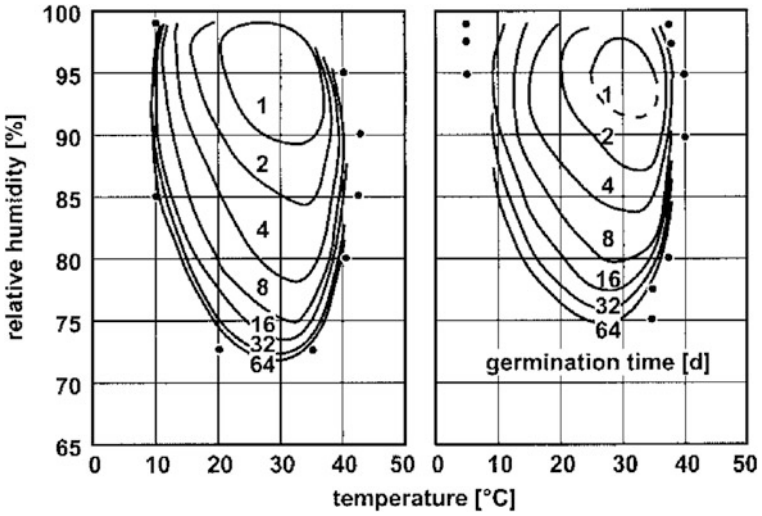

Fig. 5.2 Isopleths for mould spores of *Aspergillus restrictus* (*left* side) and *A. Versicolor* (*right* side) Reprinted from Sedlbauer (2002), copyright 2002 by SAGE, reprinted by Permission of SAGE

Substrate based isopleths are made in such a way that always the worst-case scenario is examined. For the mould growth on building materials the study considered a germination graph and a growth rate graph (Fig. 5.2).

5.1.2.5 Biohygrothermal Model

To make a more reliable prediction of the mould risk possible in cases of transient conditions, Sedlbauer extended his isopleth model with the Biohygrothermal model (Sedlbauer 2002). This model makes it possible to calculate the moisture balance of a spore independently of the transient boundary conditions, thus letting us consider interim drying out of the fungus spores (Fig. 5.3).

The Biohygrothermal model for predicting the germination of the spores is based on the fundamental idea that a fungus spore has a certain osmotic potential, so that spores can absorb water existing in the environment, i.e. in materials as well as in the air.

This potential is computationally described by means of a moisture retention curve. The absorption of humidity through the spore septum is described by diffusion, until certain moisture content inside the spore is reached that is needed for starting the metabolism. From this point on the fungus can regulate its metabolism, if necessary even independently of the surrounding conditions.

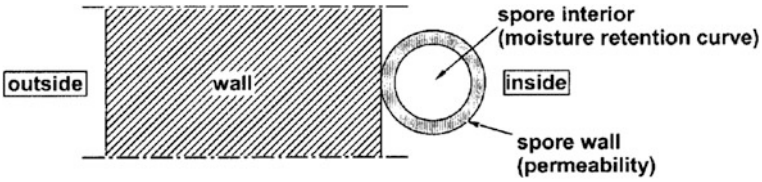

Fig. 5.3 Development of the Biohygrothermal model: wall with a mould spore (*highly enlarged*) on the inner surface. The inner surface temperature and humidity of the building wall serve as boundary conditions on both sides of the spore. Reprinted from Sedlbauer (2002), copyright 2002 by SAGE, reprinted by Permission of SAGE

Depending on the temperature, the lowest relative humidity at which the spore germination takes place can be read off the respective LIM-curves. With the help of the moisture storage function assumed for the inside spore, the corresponding critical moisture content can be calculated. Furthermore, the LIM-curves in the isopleth systems of the appropriate categories of building materials have to be used when setting the critical moisture content.

The Biohygrothermal model has been implemented in the Wufi[1] software, which includes diffusion, liquid transport and moisture storage processes.

5.2 Accelerated Experimental Testing

To preserve buildings from the colonisation of microorganisms and to act efficiently against biodeterioration, it is necessary to have a better understanding of biodeterioration mechanisms and their effects on the properties of materials. Several tests to study biodeterioration of building materials exist. Among them some were developed without accelerated weathering of the matrix leading to experiment time ranging from several month to some years, while more recently several studies have focused on the assessment of biodeterioration of building materials through laboratory accelerated ageing tests.

Laboratory tests have to be as realistic as possible. They are required to be "accelerated", reproducible, low cost and easy to implement in construction material laboratories. They also have to discriminate among the support parameters for biological growth (Escadeillas et al. 2006). The concerned topic is very broad, not only for the wide variety of materials on the market but also for the range of microorganisms.

Some examples of interesting test methods developed to date are provided in the following sections.

[1] WUFI® (Wärme und Feuchte instationär) is a software family, which allows realistic calculation of the transient coupled one- and two-dimensional heat and moisture transport in multi-layer building components exposed to natural weather.

5.2.1 Algae

Dubosc developed a general methodology for ageing tests on mortar samples (species selection and preparation, sample preparation, quantification techniques, etc...), and tested several environmental conditions which led to the design of complementary tests using different moistening modes (capillary sorption, water flow, spraying, different relative humidity levels) (Dubosc 2000). Validation tests performed on different mortar mixtures showed that some types of conditions give positive results within a 2 months period and may be used for further studies. Tests also pointed out that material porosity and roughness are very influencing factors concerning biological growth on cementitious support.

Later, together with his research group, Dubosc further developed two test methods (Escadeillas et al. 2006):

- A static test simulating growth conditions at the base of a construction. This method simulated algal growth at a wall base, where water supplies occurred by capillary ascent. It highlighted the influence of the material parameters such as the mineral composition and the pore-size distribution in the mortars. Here, algae were tested individually (one algal species per box).
- A dynamic test simulating run-off on some parts of constructions (this test corresponds to external wall surfaces exposed to rain, or leaky parts of a building or design defects). In this test, the mortar prisms were tilted at 45° in a polycarbonate transparent chamber. The faces to be studied were subjected to intermittently applied, uniform run-off of a solution inoculated with a mixture of three algae (Fig. 5.4).

Characterisation tests were performed on mortars. This choice was a simplification with respect to the initial study, which concerned concrete external walls, but in practice, the assimilation of the first few millimetres of a concrete wall to a mortar is not absurd as regards wall effect phenomena. Moreover, the use of mortars allowed small sized specimens to be made. Results showed that the algal growth between 45 and 100 days was considerable and differed according to the mortar support (Fig. 5.5).

The authors then proposed some technical methods to characterise colonised areas (Escadeillas et al. 2009). The first non-destructive method allowed a covered surface area to be estimated by image analysis (based on a clustering method). The second method, also non-destructive, allowed the density of the algae, which directly influences the stain intensity, to be estimated by spectrophotometry (or colorimetry). The third method, which is destructive and is based on the measurement of chlorophyll, was used to make a comparative quantification of the algae on various colonised mortars and to obtain information on the algal state of vitality.

Barberousse et al. used accelerated water-streaming test methods to evaluate facade coatings prone to colonisation by algae and cyanobacteria, by closely

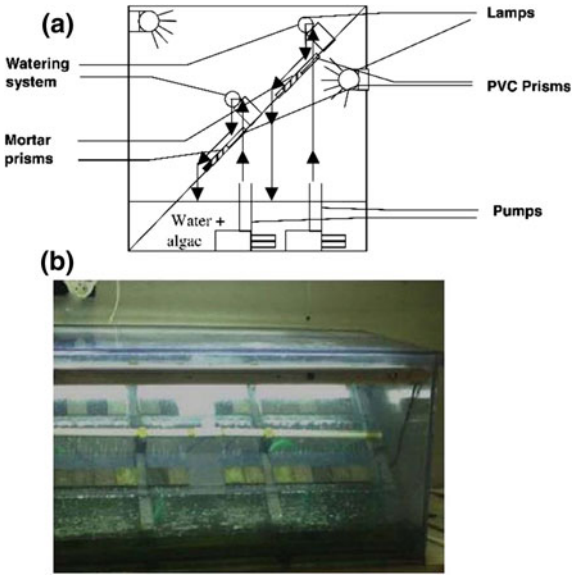

Fig. 5.4 **a** Run-off test schematic diagram. **b** Run-off test picture. Reprinted from Escadeillas et al. (2006), copyright 2006, with kind permission from Springer Science and Business Media

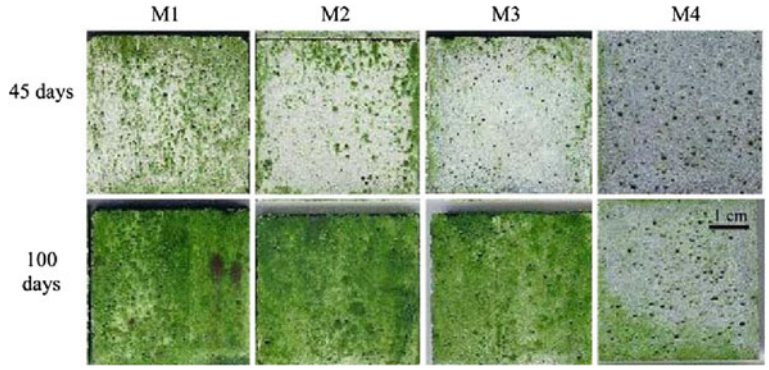

Fig. 5.5 Macroscopic aspect of mortar prisms after 45 and 100 days. Reprinted from Escadeillas et al. (2009), copyright 2009, with kind permission from Springer Science and Business Media

reproducing the phenomenon of natural biological soiling (Barberousse et al. 2007).

The water-streaming test mimics those conditions of colonisation by producing the stream of a culture of microorganisms on top of materials. The device was similar to the one previously used to investigate concrete materials (Dubosc 2000), with some improvements to investigate parameters of materials favouring algal growth.

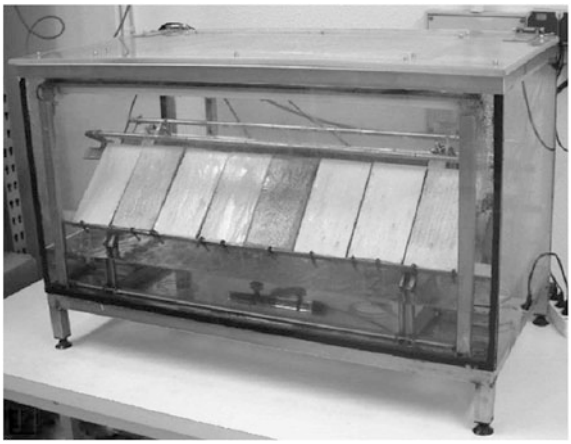

Fig. 5.6 Setup of equipment used by Barberousse et al. to verify the colonisation of the facades by microalgae and cyanobacteria. Reprinted from Barberousse et al. (2007), copyright 2007, with permission from Elsevier

The system consisted of a 100 × 50 × 50 cm glass chamber containing stainless steel supports inclined at 45° onto which specimens of facade coatings were placed (Fig. 5.6). The chamber was filled with 50 l of BBM (Bold's Basal Medium) enriched with algae or cyanobacteria cultures.

The device was equipped with two sprinkling rails made of stainless steel tubes with 2 mm diameter holes drilled at every centimetre. The rails were supplied by pumps immersed in the suspension and connected to the rails.

Thus, the principle of the water-streaming test is as follows: the suspension circulating through the sprinkling rails was directed onto the top of the specimens and ran down their surface, allowing algal and cyanobacterial cells to adhere to the surface of the specimens, depending on their characteristics, as they would in nature. The suspension was then recycled by the pumps and sprayed again onto the specimens.

The sprinkling cycles were set to start every 12 h and to run for 90 min; the amount of suspension received by each specimen of material during a cycle was $20 \pm 2\ \mathrm{lh}^{-1}$. Furthermore, since algae and cyanobacteria need light to grow, the setup also included two 30 W neon lamps placed at the same distance from the centre of the chamber: the illumination received by each specimen lasted a day length of 12 h and was set to start with the beginning of a sprinkling cycle. The glass chamber was placed in a dark room and conditioned at 23 ± 2 °C and 50 ± 5 % relative humidity.

Barberousse et al. then evaluated the colonisation kinetics of materials by image analysis. The surface of each specimen exposed to colonisation was digitised weekly using an office scanner. The obtained numerical image was treated to establish a histogram of the number of pixels versus their intensity.

The colonisation process results obtained reproduce algal growth often observed on building envelopes. This confirms that the principle of the device, by wetting the sloped materials, mimicked the humidification of facades by liquid water, with the difference that in accelerated tests the materials are inclined and the

sprinkling solution flow is high in order to accelerate the colonisation process. Porosity and roughness of the materials showed to be parameters of great influence on algae and cyanobacterial establishment.

De Muynck et al. designed a modular setup for accelerated water run-off tests, which allowed both simultaneous and separate evaluation of 12 different surface treatments for preventing algal fouling on white architectural and autoclaved aerated concrete (De Muynck et al. 2009). The modular setup consisted of 12 stainless steel compartments supported by a wooden frame at 45° inclination (Fig. 5.7).

Each compartment was equipped with a sprinkling rail on top and a plastic gutter at the bottom. A transparent 2 l PET bottle beneath each compartment served as reservoir for the algal cultures. Circulation of the algal cultures was achieved by means of an aquarium pump (200 lh^{-1}) immersed in the PET bottle. Algal cultures were introduced at the top of the compartment by means of a plastic tube connected to the sprinkling rail. Water running down from the specimens was subsequently collected by means of the gutter and a funnel covering the PET bottle.

The run-off period was set to start every 12 h and ran for 90 min. Furthermore, the setup was submitted to a 12 h day and night regime, which started simultaneously with the run-off periods. During the day regime, light was provided by means of 30 W lamps. The temperature and relative humidity ranged between 19.5 °C (night) −21.5 °C (day) and 86 % (day) −93 % (night), respectively.

Every week, the contents of the reservoirs were replaced by new algal cultures, after cleaning of the reservoirs. Additionally, every 2 weeks, the reservoirs were replaced by new ones.

The use of a modular setup also allowed testing of several biocidal treatments at the same time. In this way, leaching of biocidal compounds did not have any influence on the performance of other treatments as would be the case with

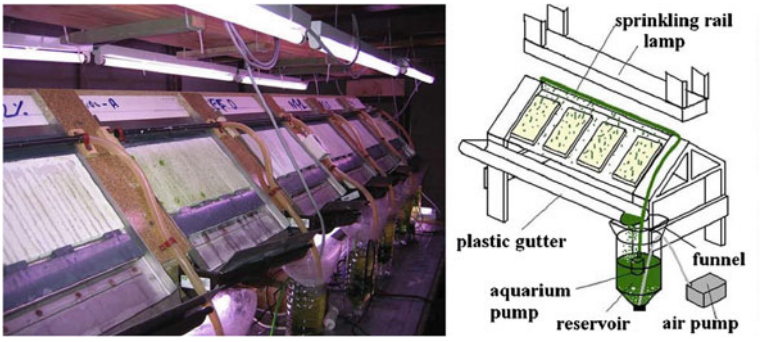

Fig. 5.7 Modular setup used for the accelerated fouling of building materials by means of algae. The image on the right gives a schematic presentation of 1 unit. Reprinted from De Muynck et al. (2009), copyright 2009, with permission from Elsevier

previous setup (Barberousse et al. 2007), where only one algal reservoir was used for all the specimens belonging to different treatments.

Furthermore, due to the weekly replacement of the algal cultures, the contribution of leached compounds on the overall performance of the treatment was greatly decreased.

Algal fouling on samples was evaluated by means of colorimetric and image analysis, for which the authors proposed new evaluation criteria, based upon a colour threshold in the CIELab[2] colour space (Fig. 5.8).

Results showed that for white concrete, contrary to untreated specimens which had 40 % of the surface covered with algae, no fouling was observed for surface treated specimens after 12 weeks of exposure to algae under test conditions. For autoclaved aerated concrete (AAC), the different strategies examined were unable to completely prevent the algal fouling. The use of water repellents resulted in green algal streaks along the surface. Biocide treated specimens showed a delay of onset of fouling of 2–4 weeks under the test conditions. Combinations of water repellents and biocides appear to be the most effective treatments for the prevention of algal fouling on concrete with a high bioreceptivity.

5.2.2 Mould

Shirakawa et al. developed and standardised an accelerated laboratory test for detecting bioreceptivity of indoor mortar to fungal growth (Shirakawa et al. 2003).

To determine which fungal species were predominant under field conditions, they used mortar samples collected from 41 buildings in two cities of Sao Paulo State in the South East of Brazil.

Then four different mortars, two laboratory-manufactured mortars composed of ordinary Portland cement, high calcium hydrated lime and standardised sand, and two different ready-mixed building mortars from the Brazilian market, were investigated for their susceptibility to colonisation by *C. sphaerospermum.*

Each of the tested mortar samples was aseptically inoculated with 25 µl of a spore suspension by placing a droplet in the centre of mortar specimen.

Large mortar samples were exposed to three different RH levels (75, 85 and 100 %), generated using saturated solutions of NaCl, KCl and pure water, respectively. Tests at 85 and 100 % RH were carried out with fungal inocula. Each mortar specimen was set in a tightly closed glass flask (600 cm^3). After inoculation, samples were incubated at 25 °C for 30 days. Interaction of *C. sphaerospermum* with mortar specimens was studied using techniques of scanning and

[2] A Lab colour space is a colour-opponent space with dimension *L* for lightness and a and b for the colour-opponent dimensions, based on nonlinearly compressed CIE XYZ colour space coordinates. CIE coordinates are based on a cube root transformation of the colour data.

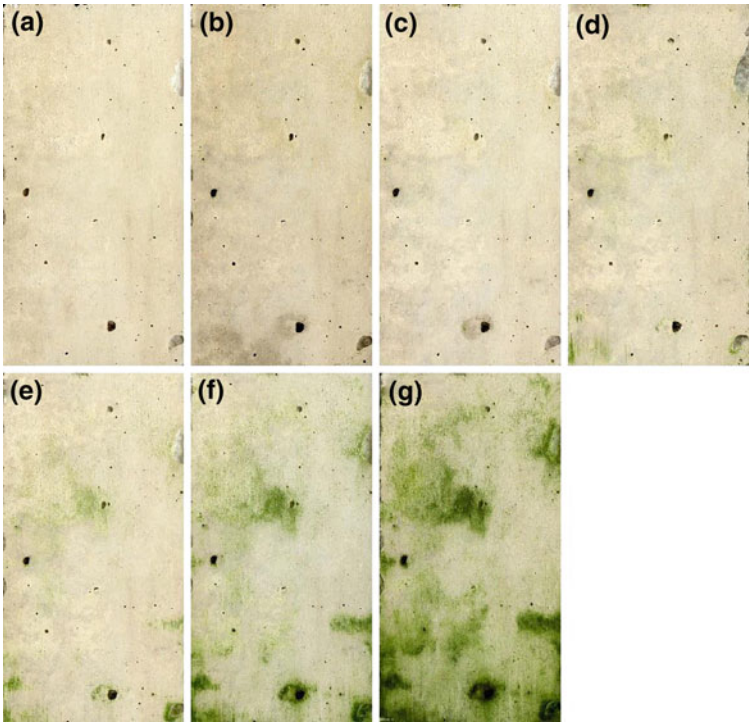

Fig. 5.8 Evolution of the visual appearance of the untreated white concrete specimens after 0 (**a**), 2 (**b**), 4 (**c**), 6 (**d**), 8 (**e**), 10 (**f**) and 12 (**g**) weeks of accelerated fouling tests. Reprinted from Muynck et al. (2009), copyright 2009, with permission from Elsevier

environmental scanning electron microscopy combined with energy dispersive X-ray analysis.

The application of the test demonstrated differences in the bioreceptivity of the four types of mortars, as revealed by light and electron microscopy studies. Parameters such as the type of substratum for casting mortars, the size of test specimens, degree of mortar carbonation and relative humidity to which mortar samples were exposed, proved to be key factors influencing the fungal bioreceptivity of mortars.

Also Urzì and De Leo carried out experiments in laboratory conditions, as well as outdoors, with artificially infected mortars to study the effectiveness of water repellents and biocides (Urzì and De Leo 2007). In the first case, the efficiency of treatments was tested against a massive colonisation of different kinds of microorganisms (bacteria, fungi and a mixture containing cyanobacteria, algae, bacteria and fungi). In the second set of experiments, under outdoor conditions, the authors observed the natural settlement of airborne microbiota on untreated and treated mortars, exposed in the city of Messina (Italy), facing North East and kept inclined at an angle of 45°.

During laboratory tests, untreated and treated mortar probes were inoculated in duplicate with fungal, bacterial and algal suspensions separately, and maintained in constant humid condition at room temperature and day light.

The progression of microbial colonisation was monitored through stereomicroscopic observations at intervals of 1 month. Fifteen months after the inoculation, all the experiments were stopped and one replica of each mortar probe was utilised for light and epifluorescent microscopic analysis and for microbiological analysis.

In both types of experiments, it was clearly shown that water repellents alone do not stop microbial colonisation, while water repellents plus biocides prevent microbial growth.

In addition, it was shown under indoor and outdoor conditions that fungi are able to colonise untreated mortars as well as those treated only with hydrophobic compounds before phototrophic microorganisms.

In a study by Wiktor et al., an accelerated laboratory test was developed to study the biodeteriorative effect of different fungal strains to a cementitious matrix (Wiktor et al. 2009).

The authors used polyethylene boxes 9.5 × 9.5 × 9.5 cm, covered by vermiculite in order to keep humidity inside. A paper sheet was disposed on it to avoid the direct contact between specimens and vermiculite. Two specimens of each matrix of ordinary white Portland cement (water/cement mass ratio 0.55) were placed in each box (Fig. 5.9).

Three fungal strains were selected for the test in order to represent the main kind of fungi involved in biodeterioration in natural environment: *Alternaria alternata* (*MC342*) to represent a hyphomycete, melanin producer *Exophiala sp.* (*MC843*) for yeast-like fungi, and *Coniosporium uncinatum (MC557)* as meristematic fungi.

Six specimens were inoculated with 1.5 ml of fungal units suspension (two unweathered, two carbonated, two carbonated and leached), and placed in three

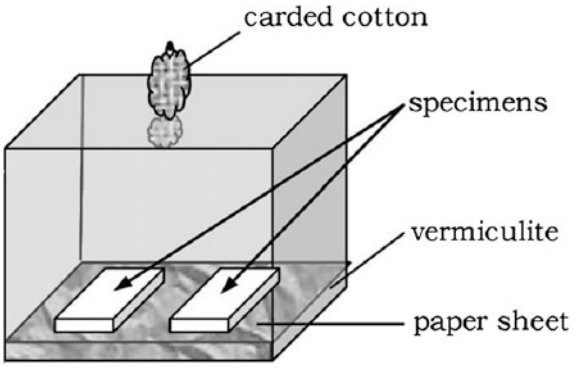

Fig. 5.9 Experimental setup for biodeterioration test. Reprinted from Wiktor et al. (2009), copyright 2009, with permission from Elsevier

different boxes, control samples were inoculated with 1.5 ml of sterile medium. Boxes were incubated at 26 °C.

In order to monitor fungal growth during the test, the surface of each specimen was observed once a week with stereomicroscope. After 4 weeks of incubation, one specimen of each box was taken and broken into small pieces for Periodic Acid Schiff Staining (PAS) and Scanning Electron Microscopy (SEM). The test permitted to obtain rapid fungal development on cement specimens.

For carbonated specimens, the growth of *Exophiala sp.* was noticed in the third week of incubation. *A. alternata* development started in the first week of incubation, and the growth increases until the fourth week. Hyphae and spore production were observed on the surface of the specimens. Development of *C. uncinatum* was noticed in the second week of incubation and increased until the fourth week.

Stereomicroscopy observations showed that microbial growth was noticed only on the surface of specimens, while PAS staining revealed the real extent of microbial growth on and within the matrix as later confirmed by SEM observations of cross section showing the penetration of hyphae inside the matrix.

Decrease in surface pH increases matrix bioreceptivity considerably. Microbial colonisation was observed on some carbonated specimens and on all carbonated and leached specimens. Carbonation is in fact the most common chemical reaction influencing cement-based materials in natural environmental scenario. That is why accelerated weathering of matrix is generally performed by carbonation (Dubosc 2000; Shirakawa et al. 2003).

Only 3 months of experiments were needed to obtain the first results—mainly related to aesthetical biodeterioration, which is rather shorter than other tests developed to date to study fungal biodeterioration.

D'Orazio et al. analysed the growth rate of three species of mould (*A. versicolor, P. chrysogenum, Stachybotrys chartarum*) on plasters, finishes and paints typically used in heavy weight building envelopes, in order to assess the influence of the substrate chemical composition (in terms of organic fraction of the materials) on the growth rate of moulds (D'Orazio et al. 2009).

The selected materials were two types of plaster (*A*, *B*), three finishes (*C*, *D*, *E*) and two paints (*F*, *G*) so as to consider different types of support belonging to the classes indicated by Sedlbauer (2001).

Two hundred microliters of each spore suspension were inoculated onto the surface of the various tested materials. All the inoculated caps were then incubated in a climatic room that could guarantee constant environmental conditions of 25 °C temperature and 95 % RH. After an incubation period of 2 weeks in the climatic chamber, the development of moulds on the different materials tested was assessed (Fig. 5.10). In addition to a naked eye comparison, a laser fluorescence microscope was used to reveal the presence of the moulds which had developed on the surface of the specimens under study. Results showed a good correlation between the quantity of organic substances contained in paints, plasters, and finishes and the growth rate of the mould.

Fig. 5.10 Mould formation (*S. chartarum*) visible to the naked eye, over a sample of an interior organic paint, after an incubation period of 2 weeks in the climatic room. Reprinted from D'Orazio et al. (2009), copyright 2009, with permission from Elsevier

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