

MOLD GROWTH ON SPRUCE AND PINE SAMPLES IN STEADY STATE CONDITIONS: TIME OF GERMINATION AND MAXIMUM COVERAGE

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ABSTRACT. Wood is a material that is environmentally friendly and has minimal carbon impact. The durability of wood depends on ambient environmental conditions. This article deals with mold growth on wood samples under constant relative humidity (75 %; 87 %; 95 % RH) and temperature (23 °C). Pine and spruce samples with surfaces oriented along the three principal anatomical directions of wood were prepared. The occurrence of mold was studied by regular microscopic observations. The mold consortium was a mixture of *Penicillium sp.*, *Aspergillus sp.*, and *Alternaria sp.*

The first signs of mold growth on pine were observed after 7 days for 87 % RH and 12 days for 95 % RH, regardless the surface orientation. Molds on spruce began to grow in 12 to 26 days at 95 % RH in dependence on surface orientation: at first on tangential surface, then on transversal surface, and finally on radial surface. The lower relative humidity of 87 % resulted in a shorter germination time: 7 days for tangential and radial surfaces, and 21 days for transversal surface.

KEYWORDS: Wood, mold, germination, spruce, pine, *Penicillium sp.*, *Aspergillus sp.*, *Alternaria sp.*

1. INTRODUCTION

Timber construction elements become more common and popular in building sector. People in many cases prefer the use organic materials instead of more energy and carbon intensive mineral-based materials. They also appreciate the benefits of fast and variable construction of wooden houses.

Wood is composed of two major elements: lignin (18–35 %) and carbohydrates (cellulose and hemicellulose, 65–75 %). The cellulose microfibrils are organized in a matrix of amorphous hemicellulose and lignin [1]. The chemical composition of wood is approximately 50 % carbon, 44 % oxygen, and 6 % hydrogen [2]. The chemical composition predetermines the mold risk of wooden material. Another factor is the nutrient content that is related to a wood specie [3]. Typical mold fungi found in moisture-damaged wood are *Alternaria alternata*, *Aspergillus sp.*, *Aureobasidium pullulans*, *Cladosporium cladosporioides*, *Chaetomium globosum*, *Paecilomyces variotii*, *Penicillium sp.*, and *Trichoderma viride* [4].

Molds on the surface of a construction element cause its aesthetic degradation. It may also allow colonization of other biodegrading agents, such as bacteria and wood-decaying fungi, and cause human health problems. The occurrence of mold also points to moisture-related problems within the construction.

The live cycle of fungi consists of three main stages: germination of spores, growth, and sporulation of hyphae. It necessitates the availability of nutrients,

water, and the right temperature. Nutrients are usually available by minor contamination of the surface by dust or other pollutants. The optimum relative humidity for mold growth is in the range of 75–98 % RH and the optimum temperature is between 10 and 40 °C, depending on the mold specie.

Humidity conditions suitable for mold growth can also be expressed on the basis of moisture content of the substrate material. Moisture content is linked to the surrounding relative humidity via the sorption (hygroscopic range), but it is also able to reflect the presence liquid water (over-hygroscopic range), and the moisture history of the substrate material.

Water can be present in wood in three forms: as a bound water within the cell walls (hydrogen bonding with accessible hydroxyl groups), and as a water vapor or liquid water in the pore system (lumina and vessels). Under steady state conditions (constant temperature and relative humidity), and in the absence of liquid water, an equilibrium occurs between the bound water and water vapor [5]. A set of these equilibrium states forms a sorption isotherm. Hygrothermal equilibrium with ambient air is not always achieved in reality of building structures. Therefore, humidity conditions suitable for mold growth should be better expressed by moisture content of the substrate material instead of relative humidity of ambient air.

This paper presents the first results of the project on fungal growth on wood exposed to changing boundary conditions. As a starting point, this paper presents and discusses the results of steady-state mold growth

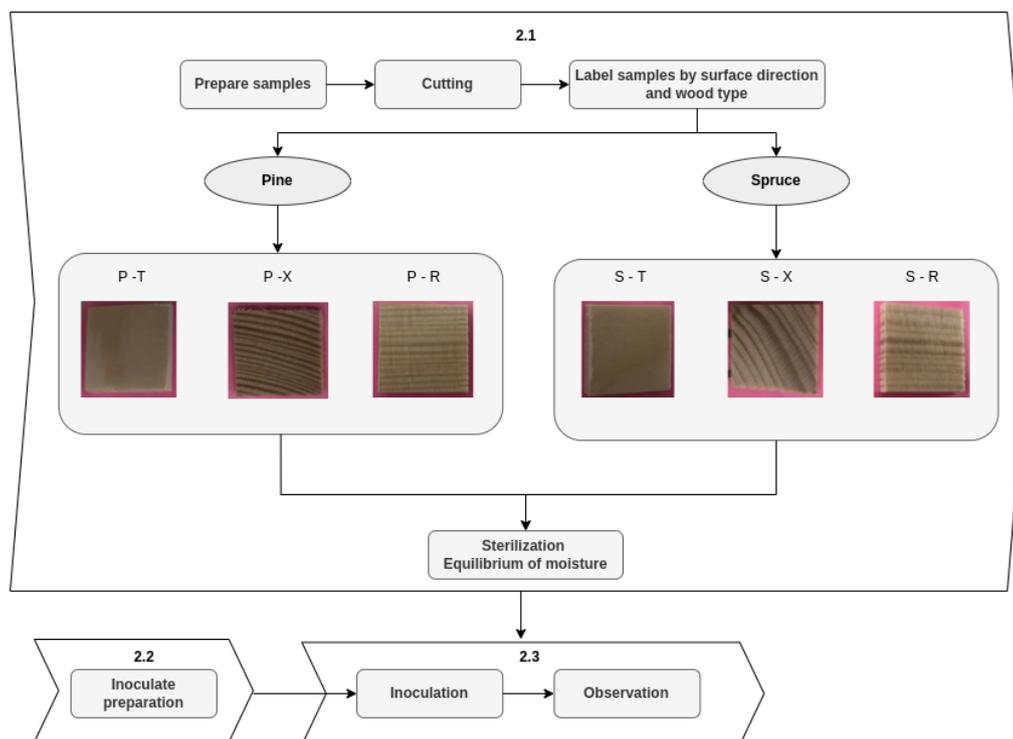


FIGURE 1. The description of experiment procedure.

experiments. The germination time and time for maximum mold coverage are presented for three levels of relative humidity. Wood samples prepared with respect to the principal anatomical directions were used.

2. METHODS AND MATERIALS

The procedure is described in Figure 1. The procedure followed the steps: sample preparation, moisture equalization, inoculate preparation, inoculation, and fungal growth observation.

2.1. PREPARATION OF SAMPLES

The samples were prepared from spruce and pine wood of commercial origin and without additional biocidal treatment. The samples ($15 \times 15 \times 3 \text{ mm}^3$) were cut so that the surfaces were oriented along the principal anatomical directions of wood. The samples were labeled using the first letter of wood species (spruce – S; pine – P), and the letter representing the observed surface (transverse surface – X; radial surface – R; tangential surface – T), see examples in Figure 1. The particular types of samples were prepared in three replications. The samples were sterilized by UV radiation – each surface was exposed to UV for 30 min. The desiccators were sterilized with ethanol, glutaraldehyde, and UV radiation. The sterile samples were placed in a sterile desiccator with the defined RH created by saturated salt solutions (75 % by NaNO_3 , 87 % by Na_2SO_4 , 95 % by KNO_3). The samples were kept there for 14 days to reach the equilibrium moisture content. Prior to the sterilization

and conditioning, the samples were stored in a stable environment with RH lower than 60 %.

2.2. PREPARATION OF INOCULUM

The inoculum fungi were selected from common occurrence genera in the Czech Republic. The most common molds identified in the Czech Republic are *Alternaria sp.*, *Cladosporium sp.*, *Aureobasidium sp.*, *Penicillium sp.*, *Fusarium sp.*, and *Aspergillus sp.* [6]. The particular molds chosen for the experiments were *Aspergillus niger* (CCM 8155), *Penicillium brevicompactum* (CCM 8040), and *Alternaria alternata* (CCM F-397). These genera were selected because of their ability to grow on wood samples, good sporulation, and easy microscopy observation.

The molds gained from Czech collection of microorganism were cultivated triple on Czapek Dox agar at $23 \pm 2^\circ\text{C}$ for seven days. The spores were collected by the following procedure: The agar plate, which was covered with sporangia mold, was poured over with 1 ml of sterile saline buffer. The spores were loosened with a sterile glass rod. The solution with spores and debris of hyphae was taken by sterile pipette and filtrated by sterile gauze with a suitable mesh size. The particular mold genera were collected separately and the spore concentrations were calculated by Buchner chamber under a microscope with magnification from $10\times$ to $40\times$. The suspension of the particular mold spores was adjusted to $1.1 \cdot 10^5 \text{ CFU/ml}$. The inoculum was prepared as a mixture of particular spores in the ratio (1:1:0.5; *Penicillium purpurogenum* : *Alternaria alternata* : *Aspergillus niger*).

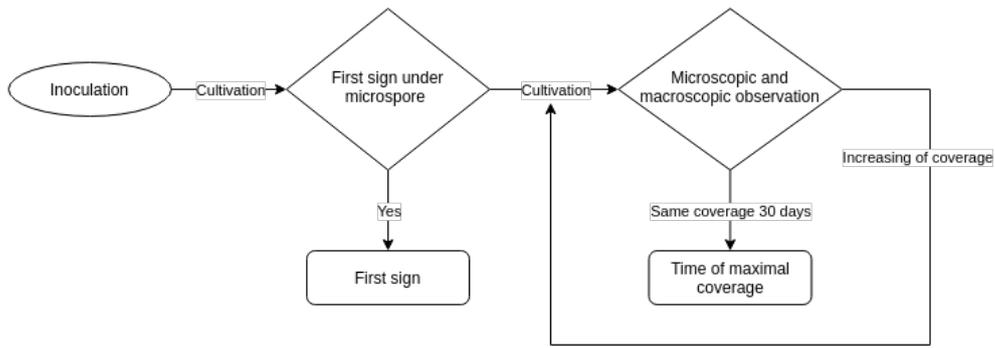


FIGURE 2. First sign of mold growth and maximal coverage.

Label	P-X			P-T			P-R			
	RH [%]	75	87	95	75	87	95	75	87	95
First sign [day]	-	7	12	-	7	12	-	7	12	
Max coverage [day]		26	34		46	34		68	21	

TABLE 1. Time in days required for the first signs of mold growth and the maximum coverage of pine samples.

2.3. INOCULATION OF SAMPLES AND OBSERVATIONS

After initial 14-day long exposure with the selected RH, the samples were inoculated by spraying a volume of 200 μl containing the mixture of molds as prepared by the procedure described in Section 2.2.

The inoculated samples were placed in sterile desiccators maintaining 75 %, 87 %, and 95 % RH at 23 °C. The mold growth was observed by Olympus BX41 microscope with the magnification of 40× to 400×. At the beginning of the experiment the observation was performed daily. The observation period was extended to 3 and 7 days after 14 days from the beginning.

Two significant states were defined and times of their achieved since the beginning of the experiment (inoculation) were monitored:

The first sign – is the time when hyphae or sporangium was first observed under microscope with 100× magnification

Maximum coverage – time since which no more molds have grown for one month. One month was set as an opportunity to observe the germination of spores newly formed during the experiment; see Figure 2.

The total duration of the experiment was 3 months, and subsequently, the experiment had been repeated twice to obtain more dates.

2.4. THE DETERMINATION OF GERMINATION ON AGAR

The control of viability and size of hyphae and sporangium was made on agar by follow procedure. The agar samples were made on a microscope slide following the procedure. The nutrient agar was made from a commercial dry mixture (Czapek Dox Agar, Sigma-Aldrich, USA) according to their protocol on Petri dishes. Nutrient agar was cut with a sterile

scalpel to size approximately 74 mm × 50 mm and placed on a microscope slide (76 mm × 52 mm). The inoculate solution was transferred to the agar surface by spraying (twice) to obtain uniform coverage of the surface by mold spores. The particular inoculate mold suspension was used in two concentrations. The “plus” concentration was $1.1 \cdot 10^5$ CFU/ml as described in Section 2.2 and the “minus” concentration was diluted in a 1:1 ratio with sterile water. The mold mixture suspension was not used, we used only particular species (*Penicillium purpurogenum*, *Aspergillus niger*). The inoculated agar samples were placed in humid conditions above water (RH close to 100 %) at 23 ± 1 °C for 14 days.

3. RESULTS

3.1. THE PREPARATION SAMPLES

The samples were made by the procedure described above and named by wood species, type surface, and surrounding RH. Control of UV sterilization was performed by agar test. The wood specimens placed on agar plate (Czapek Dox agar) for 5 days and there was no evidence of mold growth under a microscope with magnification 40–100×.

3.2. GERMINATION AND GROW ON WOODEN SAMPLES

3.2.1. PINE SAMPLES

The environment with 75 % RH was not sufficient for mold growth. There was no evidence of hyphae or sporangia. The environments with 87 % RH and 95 % RH led to mold growth, see Table 1. For all the samples, the first signs of mold growth were visible after 7 days in the environment with 87 % RH. The environment with 95 % RH prolonged the onset of

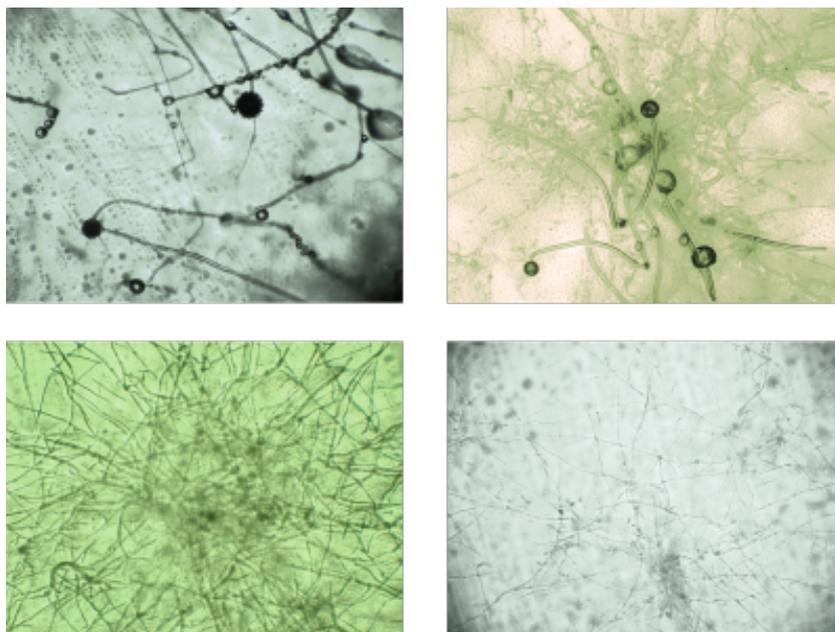


FIGURE 3. The microscopic images with magnification $100\times$ after 18 h; the first row *Aspergillus* plus and *Aspergillus* minus, the second row *Penicillium* plus and *Penicillium* minus.

Label	S-X			S-T			S-R		
RH [%]	75	87	95	75	87	95	75	87	95
First sign [day]	-	21	15	-	7	12	-	7	26
Max coverage [day]		54	61		21	34		47	34

TABLE 2. Time in days required for the first signs of mold growth and the maximum coverage of spruce samples.

mold growth to 12 days. Samples P-R-95 reached the maximum coverage in the shortest time. Samples P-X-87 needed 26 days, the samples P-X-95 and P-T-95 both needed 34 days. The P-T-87 achieved maximal coverage on the 46th day, whereas P-R-87 needed 68 days.

3.2.2. SPRUCE SAMPLES

The environment with 75% RH was not sufficient for mold growth. There was no evidence of hyphae or sporangia. The environments with 87% RH and 95% RH led to mold growth, see Table 2. Samples S-T-87 and S-R-87 showed the first signs of mold growth after 7 days in the environment with 87% RH. Samples S-T-95 showed the first signs of mold grow after 12 days. Samples S-X-95 showed the first signs of mold grows after 15 days. Samples S-X-87 needed the longest time for the first molds (21 days). Samples S-T-87 reached maximum coverage in the shortest time (21 days). Samples S-T-95 and S-R-95 needed 34 days, whereas samples S-R-87 needed 47 days. S-X-87 achieved maximum coverage after 54 days and S-X-95 after 61 days.

3.3. GERMINATION ON AGAR SAMPLES

Aspergillus niger formed visible sporangia on agar after 18 h. Samples with a higher initial spore concentration showed a higher frequency of sporangia than

samples with a lower initial concentration. *Penicillium purpurogenum* formed visible hyphae. Hyphal coverage was found to be greater in samples with higher initial spore concentration (Figure 3).

4. DISCUSSION

The samples were made from pine and spruce and the sterilization was proceeded by UV radiation to minimization of structure change in wood. The method of sterilization may has influence to mold growth by damaging of wood structure or releasing of nutrient [7], we used the sterilization with the least known influence on chemical and structural properties of wood. The control experiment showed that used sterilization time is sufficient for the killing of all debris of microorganisms, the same for spores of bacteria and mold [8–10].

The further input key factor is the compose of mold inoculum. in terms of species representation, life stage (spores, hyphae) and concentration of individual molds. The mold species were chosen due on literature revue [11–13] and based on previous projects in the laboratory [14–16].

Fungal growth was monitored between 75 and 95% RH, which is the most commonly reported humidity for fungal growth in the literature [12, 17–20] under constant optimal temperature (23 °C). The samples

were kept in particular humidity for achieved moisture balance for 14 days before main experiment. The samples had equal amount of moisture content after equalization phase regardless of origin wood species and principal anatomical directions.

Growth of the genera *Penicillium sp.* and *Aspergillus sp.* was observed on wood samples. There was no evidence of *Alternaria sp.* germination in our experiments for 3 months. The experiments used clean wood without pollutants or dust and it could be an unsuitable nutrient for growing *Alternaria sp.* Although this genus is known for them occurring on cellulose-based materials [1, 2, 18] wood materials may not be their nutrient.

The literature lacks of data on the dependence of mold growth on principal anatomical directions. and generally there is little information about the mold growth for spruce. The time of first sign of mold growth is dependent on visibility or/and observability hyphae and sporangium. The observation of hyphae on wood samples is almost very difficult or even impossible for colourless and small size of hyphae. Therefore, we called this stage the first sign of mold growth instead of mold germination. This values is quite clear and carries only a small error of one or two days at most.

The coverage rate as a mold index [21] is determined by the concentration of spores used for inoculation, which is the term in the mold growth model. For our research, the term time to reach maximum coverage is the time from which no further fungal growth increased. It is not a total coverage of the sample. Determining the exact time from when no more growth occurred is very problematic because it is a subjective assessment. The degree of uncertainty in determining this value is on the order of a week.

The tendency of first signs of mold growth is more dependence on used RH then on principal anatomical directions and wood species besides spruce samples with traversal direction. Johansson et al. [11] reported that the start of mold growth on pine is around one week for 85 % RH and two weeks for 95 % RH. This corresponds to our results which showed mold growth on the 7th day for 87 % RH and 12th day for 95 % RH for all principal anatomic direction. In our experiment, we observed a faster onset of mold growth then Johansson [11] and it can be caused by the higher used humidity, smaller samples, and *Aspergillus sp.* used in the inoculum. The onset of mold growth on spruce had same tendency for tangential and radial principal anatomic direction as pine except that for the radial direction at 95 % RH the growth was observed later. The traversal direction for spruce showed other results. This may be due to the sporangium starting to grow later or being hidden between the filaments for a longer period of time.

We determined that the pine samples achieved maximal coverage at 21st (P-R-95) to 34th (P-X-95 and P-T-95) day for 95 % RH. Our results are shorter than

reported Johansson et al. [11]. The lower RH (87 % RH) increased time required to achieved maximal coverage on pinewood. It is 26th day for P-X-87; 46th day. It may be due that the researchers made mainly experiments with samples which had similar principal anatomic directions as P-T, for the simplest preparation of a smooth sample surface. Other reasons may be the higher humidity we used, the smaller sample size and the *Aspergillus sp.* used in the inoculum. For spruce wood, the time required to achieve maximum coverage varied widely across the principal anatomic direction. The samples with the tangential and radial directions have similar leaf ring densities

We assumed that new spores would be released from the sporangia and new fungi would grow from them and therefore the coverage rate would increase in waves, but this assumption was not confirmed. It seems that the newly formed spores did not come into contact with the wood, i.e. the nutrient soil.

5. CONCLUSION

This paper presents results from an experiment that tracked mold growth on spruce and pine in relation to principle anatomical directions. Our assumption that the growth tendency would be similar for the same directions in both tree species was not confirmed. The apparent onset of fungal growth in pine depends only on ambient RH and in spruce depends not only on RH used but also on principle anatomical directions. Determining the time required for maximum coverage is very problematic and its determination is burdened with large errors. In order to be more accurate, it would be necessary to increase the number of samples on which mold growth wood be recorded on the whole sample with a resolution of at least 40 times every three days. And then to do an image analysis.

ACKNOWLEDGEMENTS

This work was supported by the grant 20-12941S the Grant agency of Czech republic and we are extremely grateful to our technical worker Ivana Loušová a Petra Šutová.

REFERENCES

- [1] A.-M. Olsson, L. Salmén. The effect of lignin composition on the viscoelastic properties of wood. *Nordic Pulp & Paper Research Journal* **12**(3):140–144, 1997. <https://doi.org/10.3183/npprj-1997-12-03-p140-144>
- [2] R. C. Pettersen. *The Chemistry of Solid Wood*, chap. 2 The Chemical Composition of Wood, pp. 57–126. American Chemical Society, Washington, 1984. <https://doi.org/10.1021/ba-1984-0207.ch002>
- [3] R. G. de Paiva, M. A. de Moraes, F. C. de Godoi, M. M. Beppu. Multilayer biopolymer membranes containing copper for antibacterial applications. *Journal of Applied Polymer Science* **126**(S1):E17–E24, 2012. <https://doi.org/10.1002/app.36666>
- [4] A. Hukka, H. A. Viitanen. A mathematical model of mould growth on wooden material. *Wood Science and*

- Technology* **33**(6):475–485, 1999.
<https://doi.org/10.1007/s002260050131>
- [5] P. A. van Meel, S. J. F. Erich, H. P. Huinink, et al. Moisture transport in coated wood. *Progress in Organic Coatings* **72**(4):686–694, 2011.
<https://doi.org/10.1016/j.porgcoat.2011.07.011>
- [6] P. Ryparová. *Stanovení antimikrobiálního charakteru materiálů s ohledem na nové trendy ve stavebnictví*. Ph.D. thesis, FCE CTU, Prague, 2017.
- [7] K. Gradeci, N. Labonnote, B. Time, J. Köhler. Mould growth criteria and design avoidance approaches in wood-based materials – A systematic review. *Construction and Building Materials* **150**:77–88, 2017. <https://doi.org/10.1016/j.conbuildmat.2017.05.204>
- [8] N. Hayashi, Y. Yagyu, A. Yonesu, M. Shiratani. Sterilization characteristics of the surfaces of agricultural products using active oxygen species generated by atmospheric plasma and UV light. *Japanese Journal of Applied Physics* **53**(5S1):05FR03, 2014. <https://doi.org/10.7567/jjap.53.05fr03>
- [9] Y. Hidaka, K. Kubota. Study on the sterilization of grain surface using UV radiation: Development and evaluation of UV irradiation equipment. *Japan Agricultural Research Quarterly: JARQ* **40**(2):157–161, 2006. <https://doi.org/10.6090/jarq.40.157>
- [10] T. Kawaguchi, A. W. Decho. A laboratory investigation of cyanobacterial extracellular polymeric secretion (EPS) in influencing CaCO₃ polymorphism. *Journal of Crystal Growth* **240**(1–2):230–235, 2002. [https://doi.org/10.1016/S0022-0248\(02\)00918-1](https://doi.org/10.1016/S0022-0248(02)00918-1)
- [11] P. Johansson, A. Ekstrand-Tobin, T. Svensson, G. Bok. Laboratory study to determine the critical moisture level for mould growth on building materials. *International Biodeterioration & Biodegradation* **73**:23–32, 2012.
<https://doi.org/10.1016/j.ibiod.2012.05.014>
- [12] P. Johansson, L. Lång, C.-M. Capener. How well do mould models predict mould growth in buildings, considering the end-user perspective? *Journal of Building Engineering* **40**:102301, 2021.
<https://doi.org/10.1016/j.jobe.2021.102301>
- [13] A. Koziróg, A. Otlewska, M. Piotrowska, et al. Colonising organisms as a biodegradation factor affecting historical wood materials at the former concentration camp of Auschwitz II – Birkenau. *International Biodeterioration & Biodegradation* **86**:171–178, 2014.
<https://doi.org/10.1016/j.ibiod.2013.08.004>
- [14] P. Ryparová, R. Wasserbauer, P. Tesárek, Z. Rácová. Preparation of antimicrobial treatment interiors using nano textiles. In *Central Europe Towards Sustainable Building 2013*, pp. 1–4. 2013. http://www.cesb.cz/cesb13/proceedings/3_materials/CESB13_1294.pdf
- [15] Z. Rácová, R. Wasserbauer, P. Ryparová. Microscopic filamentous fungi in buildings, preventing their occurrence and their remediation using nanofibers. *Advanced Materials Research* **649**:89–92, 2013. <https://doi.org/10.4028/www.scientific.net/AMR.649.89>
- [16] P. Ryparová, R. Wasserbauer, Z. Rácová. The cause of occurrence of microorganisms in civil engineering and the dangers associated with their growth. *Procedia Engineering* **151**:300–305, 2016.
<https://doi.org/10.1016/j.proeng.2016.07.402>
- [17] S. S. Block. Humidity requirements for mold growth. *Applied Microbiology* **1**(6):287–293, 1953.
<https://doi.org/10.1128/am.1.6.287-293.1953>
- [18] K. F. Nielsen, G. Holm, L. P. Uttrup, P. A. Nielsen. Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism. *International Biodeterioration & Biodegradation* **54**(4):325–336, 2004.
<https://doi.org/10.1016/j.ibiod.2004.05.002>
- [19] H. Viitanen, M. Salonvaara. *Indoor Air*, chap. Moisture conditions and biodeterioration risk of building materials and structure, (Figure 1), pp. 1001–1006. 2015. <https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.524.9841&rep=rep1&type=pdf>
- [20] T. Isaksson, S. Thelandersson, A. Ekstrand-Tobin, P. Johansson. Critical conditions for onset of mould growth under varying climate conditions. *Building and Environment* **45**(7):1712–1721, 2010.
<https://doi.org/10.1016/j.buildenv.2010.01.023>
- [21] H. Viitanen, T. Toratti, L. Makkonen, et al. Towards modelling of decay risk of wooden materials. *European Journal of Wood and Wood Products* **68**(3):303–313, 2010. <https://doi.org/10.1007/s00107-010-0450-x>