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1 Resistance to mold development assessment of bio-based building 2 materials

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13

14 Abstract

15 Nowadays, insulating building materials are developed from the valorization of agro-resources. They
16 show high ecological and hygrothermal performance. Before making them available on the market,
17 there is a need to classify them according to their decay resistance. This paper aims to propose a test
18 method that qualifies bio-based composites with respect to their performance. An accelerated aging
19 test was carried out on 5 composites made with two different agro-resources (hemp and rape) and
20 with different binders. It consists of exposing the specimens to (30 °C; 90% RH) for three months.
21 During the test, the specimens are regularly weighed and photographed. The sample mass and the
22 percentage of surface contaminated by fungi are measured along the test. Finally, a microscopic view
23 allows identifying the species of the developed molds.

24

25 Keywords

26 A. Biocomposite; B. Environmental degradation; D. Non-destructive testing; D. Optical microscopy;
27 DIC (Digital Image Correlation).

28

29 1. Introduction

30 The development of new insulating building materials from agro-resources of local agriculture (hemp
31 shiv, flax shiv, wheat straw, etc.) is currently booming. The main quality of these materials is their
32 hygrothermal performance when compared with traditional building materials. However, before they
33 can be made available in the market, others characteristics need to be evaluated such as mechanical
34 performance and durability. This paper investigates the decay resistance of the newly developed
35 composites. Bio-based materials are assumed to be highly sensitive to the mold growth due to their
36 chemical composition: cellulose, hemicellulose, lignin and proteins.

37 The molds are produced by wide variety of microorganisms, mainly microscopic fungi and yeasts.
38 These play an important role in the decomposition of plant materials. These microorganisms grow and
39 reproduce very quickly, spreading spores and mycelium.

40
41 These microorganisms may already be present inside building materials (walls, partitions, ceilings) or
42 may be directly introduced into homes through ventilation. Their growth depends on various factors, in
43 particular: flood, water leaks (roof or pipework), poorly-ventilated spaces (in kitchen, in bathroom) and
44 building seals [1,2]. In all cases, the fungi will grow when materials contain free water. For wood, the
45 water content at the fiber saturation point ranges from 20 to 30 %. The appearance of fungi induces a
46 mass loss of between 5 and 10 % and the mechanical properties decrease between 20 and 80 %
47 depending on the type of material [3]. The molds collect water, nutritional substances and minerals
48 needed for their synthesis from the surrounding environment and the materials [2,4–7].

49
50 Although fungi are present in the atmosphere, higher concentrations may result in adverse health
51 effects. Indeed, many fungal components are likely to cause adverse effects on the health of exposed
52 individuals. Mycelial fragments can be present in ambient air and could be inhaled. Their walls contain
53 glucans (complex sugar) with inflammatory properties. Spores may also cause allergic reactions such
54 as allergic rhinitis or worsening of asthma symptoms. Furthermore, fungi release toxins of which
55 toxicity may persist after the fungi have ceased to grow. The threshold of sensitivity varies from one
56 individual to another, and according to the type of mold for each person. The individual response and
57 the experienced symptoms vary considerably depending on the individual sensitivity and the duration
58 of exposure to the fungi. Infants, children, the elderly, pregnant women and people suffering from

59 respiratory diseases, allergies, asthma and weakened immune systems are usually more sensitive to
60 mold exposure. Likewise, the severity is not necessarily proportional to the extent of exposure. Mold
61 prevention and remediation in new and existing housing is thus not solely an aesthetic issue, but also
62 one of public health and hygiene [1,5,8,9].

63
64 There is therefore a need to classify materials depending on their decay resistance in order to use
65 them appropriately. To qualify the durability of materials, different methods and standards exist such
66 as: Johansson method [10], AWWA Standard E24-06 [11], ASTM 2012 [12] or BSI EN ISO 846 – 1997
67 [13], assessing the resistance of specimen surface to mold growth after fungi inoculation. For each of
68 these methods, the intensity of specimen's mold growth is visually rated using a scale during the test
69 period. Thus, this is a somewhat subjective type of assessment.

70
71 The aim of this paper is to develop a method to qualify bio-based composites with respect to their
72 resistance to mold development. The composites were exposed over a three months period to
73 unfavorable conditions: 30°C and 90% RH. These conditions are expected to initiate and accelerate
74 mold growth on the surface of bio-based panels. The composite specimens were made with two
75 different agro-resources (hemp and rape) mixed with different binders. They are tested without prior
76 sterilization or inoculation with mold. Specimens are regularly weighed and photographed during the
77 test. The evolution of two indicators is thus measured over time: the sample mass and the percentage
78 of its surface contaminated by fungi. Two ways are used to rate the contaminated area: visual
79 inspection as stated previously and digital image correlation (DIC). The latter method is developed in
80 this work for quick quantification of the contaminated surface of the specimens by molds in an
81 objective manner unlike the visual evaluation. At the end of the test, microscopic views are analyzed in
82 order to identify the mold species which have contaminated the composite.

83

84 **2. Materials and methods**

85 **2.1. Bio-aggregates**

86 Two types of aggregates are considered in this study: hemp shiv and rape straw (Fig. 3). The main
87 characteristics of these aggregates are reported in Table 1. The particle size distribution, bulk density
88 and thermal conductivity were obtained using protocols set by RILEM TC 236-BBM [14]. The moisture

89 buffer value (MBV) is measured for bulk materials according to a method based on the NORDTEST
90 protocol [15].

91

92 The hemp shiv is a commercial product (Biofibat – CAVAC, France) commonly used to produce hemp
93 concrete. The mean width of particles (W50) is 2.2 mm and the mean length (L50) is 8.0 mm. The
94 maximal width is 5.0 mm and the maximal length is 19.0 mm. The thermal conductivity is 64.1
95 mW/(m.K) for bulk density around of 110 kg/m³. The MBV is 2.35 g/(m².%RH). According the
96 classification of the Nordtest project, bulk hemp shiv appears as an excellent hygric regulator (MBV
97 higher than 2 g/(m².%RH)).

98

99 The rape straw (supplied by CAVAC, France) is the residual portion of the threshing of rape commonly
100 used for animal bedding, mulching or animal feeding. The mean width of particles is 3.1 mm and the
101 mean length is 15.9 mm. The maximal width is 8.5 mm and the maximal length is 45.2 mm. The
102 thermal conductivity is 49.9 mW/(m.K) for bulk density around of 75 kg/m³. The MBV is 2.25
103 g/(m².%RH). According the classification of the Nordtest project, it is an excellent hygric regulator
104 (MBV higher than 2 g/(m².%RH)).

105

106 *Table 1 : Main characteristics of hemp shiv and rape straw*

Aggregates	Hemp shiv	Rape straw
Mean Width (W50, mm)	2.2	3.1
Maximal width (mm)	5.0	8.5
Mean Length (L50, mm)	8.0	15.9
Maximal length (mm)	19.0	45.2
Bulk density (kg/m ³)	107.9	73.3
Thermal conductivity (mW/(m.K))	64.1	49.9
MBV (g/(m ² .%RH))	2.35	2.25

107

108 **2.2. Composite formulations**

109 The composite formulations are developed within ISOBIO project [16]. During this project, several
110 lines of research are studied with the aim of producing, from these bio-aggregates and a green binder,
111 a rigid insulation panel. The main lines of research are as follows:

- 112 ▪ Use the adhesive properties of aggregates after treatment,
- 113 ▪ Use crosslink binders to improve the mechanical properties and water repellence of
- 114 composites,

- 115 ▪ Use a sol-gel treatment to improve the water repellence of composites.

116

117 Numerous formulations were investigated and the five best ones are selected to produce specimens
118 for this study. They are made of rape straw or hemp shiv, treated or not, mixed with or without binders
119 and with or without additives. The formulations are summarized in Table 2.

120

121 C1 composites are made of rape straw after exposure to alkali treatment. Preliminary work showed
122 that after alkaline and thermal treatments on rape straw, the produced composites have good
123 cohesion, which is induced by the polymerization of soluble components. This formulation has an
124 advantage to only use treated rape straw and thus 100 % bio-based insulation panels can be
125 produced in the same line as the hemp-straw composites [17].

126

127 C2 composites are made of hemp shiv mixed with a binder formulated using a starch derivative and a
128 crosslinker. The starch and its derivatives have good adhesive properties on the lignocellulosic
129 substrates. However an important issue is the low energy chemical bonds (Van der Waals and
130 hydrogen bonds), which ensure starch cohesion. Moreover, they can be easily hydrolyzed by water,
131 resulting in poor water resistance of the composite. Therefore a crosslinker is added to create, after a
132 thermal activation, covalent bonds between the starch macromolecules chains and thus, to form a
133 three-dimensional network in order to improve the mechanical strength and the water resistance of the
134 composite. C3 composites are made using the same formulation as C2 composites, but 2 % of
135 paraffin is added, to improve its water repellence.

136

137 C4 composites are made of hemp shiv coated with a sol-gel treatment to improve their water
138 repellence. For the preparation of the sol-gel, 1 M of TEOS was added to a mixture of 4 M distilled
139 water, 4 M of absolute ethanol and 0.005 M of nitric acid. 0.015 M of HDTMS was added to the above
140 mixture as the hydrophobic agent. The sol was vigorously stirred at 40 °C and atmospheric pressure
141 for nearly 2 hours. The sols were allowed to age for 96 hours in closed container at room temperature
142 before to be mixed with the hemp shiv in order to coat them. In the case of C4 composites, this sol-gel
143 coating also serves as a binder. C5 composites are made of the same coated aggregates as C4

144 composites and the same binder as C2 composites. Compared to C4 composites, the addition of the
 145 binder improves the mechanical properties.

146

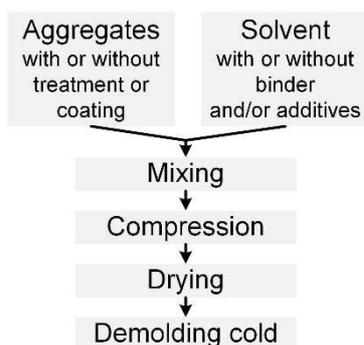
147 *Table 2 : Specimens composition*

Formulations	C1	C2	C3	C4	C5
Aggregates	Rape straw 100 %	Hemp shiv 90 %	Hemp shiv 88 %	Hemp shiv 100 %	Hemp shiv 90 %
Treatment	Alkali	Without	Without	Sol-gel	Sol-gel
Binder	Without	8% Starch derivative 2 % Crosslinker	8% Starch derivative 2 % Crosslinker	Without	8 % Starch derivative 2 % Crosslinker
Additive	Without	Without	Paraffin 2%	Without	Without
Solvent	Without	Water 33 % of dry content	Water 33 % of dry content	Without	Water 33 % of dry content

148

149 2.3. Composite production process

150 For preparation of C1, C2, C3 and C5 composites, the bio-aggregates (treated, coated or not) are
 151 moistened with the solvent and/or the binder, if any. Three specimens are produced from the same
 152 mixture. This batch is divided into three equal parts (A, B and C) and each part is introduced in one of
 153 the three cells of a mold. Each part is compacted 5 times at 0.25 MPa in the mold and then placed in
 154 an oven at 190 °C for 2 hours. The three specimens of dimensions 100 x 100 x 50 mm³ are demolded
 155 once they cool down to room temperature (Figure 1).



156

157 *Figure 1 : Flow-chart of composites production (except C4 formulation)*

158

159 For C4 formulation, mixing of the constituent materials, hemp shiv (75 vol%) and sol (25 vol%), were
 160 carried out manually to achieve a uniform mixture. The mass of the materials was pre-calculated to
 161 target a final density of 175 kg/m³ for the composites. Hemp shiv were mixed with the sol and then
 162 placed into a phenolic ply mold, tamped down and left for overnight in the oven at 80 °C. The

163 specimens of dimensions 100 x 100 x 50 mm³ were removed from the molds and transferred to a
 164 conditioning room at 19 °C and 50% RH.

165

166 Figure 2 shows the produced composites and Table 3 gives their apparent density. The composites
 167 based on hemp shiv have very close densities ranging from 173 to 203 kg/m³. The composite based
 168 on rape straw (C1) has the highest density (288 kg/m³) due to much higher density of the aggregate
 169 after alkali-treatment which reduces intra and inter-particle porosity. The C1 composites have the
 170 highest pH (10) due to alkali treatment. The composites made with starch derivative based binder and
 171 crosslinker (C2, C3 and C5), have an acid pH of 6. The composites which are glued with sol-gel (C4),
 172 have the lowest pH (4).

173



174

175 *Figure 2 : Developed composites*

176

177 *Table 3 : Apparent density and pH surface of composites*

N°	C1	C2	C3	C4	C5
Density (kg/m ³)	287.66 ± 9.49	181.02 ± 2.82	184.21 ± 1.51	173.92 ± 6.11	203.10 ± 4.20
CoV	3.30 %	1.56 %	0.82 %	3.51 %	2.07 %
pH	10	6	6	4	6

178

179

180 **2.4. Methods for characterization**

181 **2.4.1. Biological aging**

182 The accelerated aging test evaluates the fungal resistance of the composites at given temperature
 183 and humidity and is schematized in Figure 3. After stabilization at 23°C and 50% RH, the biological
 184 aging tests are performed in a climate chamber (Vötsch VC0034, Figure 3.1) under controlled
 185 temperature and relative humidity at 30°C and 90% RH for three months. The specimens are tested
 186 without prior sterilization or inoculation with mold to closely reproduce a real exposure situation.

187

188 For each formulation, three specimens are tested. Each specimen (3 specimens per formulation) is
 189 placed in a plastic box to prevent contamination between specimens. In order to facilitate image
 190 treatment, the specimen is prevented from moving in the box, by making use of the aluminum
 191 adhesive block. Furthermore, the experimental bench allows to take pictures of the top surface of the
 192 specimens in a consistent manner (Figure 3.3). Every week day, the specimens are weighed (Figure
 193 3.2) and visually inspected. Pictures are captured with a camera (Coolpix 8700, 8 Megapixels, Nikon).

194
 195 For the analysis, mass variation from the steady state point is related to the exchange surface area
 196 (including horizontal top and lateral surfaces of the specimen). The mass loss of each formulation is
 197 expressed as the mass ratio between the maximal mass and this of the given time, using Equation (1).

$$198 \quad ML = \frac{m_{max} - m}{m_{max}} \times 100 \quad (1)$$

199 Where ML is the mass loss (%); m_{max} is the maximal mass of composite (g) and m is the mass of
 200 composite at the given time (g).

201
 202 On the final day of the test, the samples are visually evaluated for mold growth according the rating
 203 scale of BSI EN ISO 846 – 1997 [13], which is shown in Table 4. This method is subjective. Thus, the
 204 final rating is the average value of the given rating by each author in this publication, making a total of
 205 8 people.

206

207 *Table 4: Assessment of growth following the BSI EN ISO 846 – 1997 [13]*

Intensity of growth	Evaluation
0	No growth apparent under the microscope.
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, covering up to 50 % of the test surface.
4	Considerable growth, covering more than 50 % of the test surface.
5	Heavy growth, covering more than 75 % of the test surface.

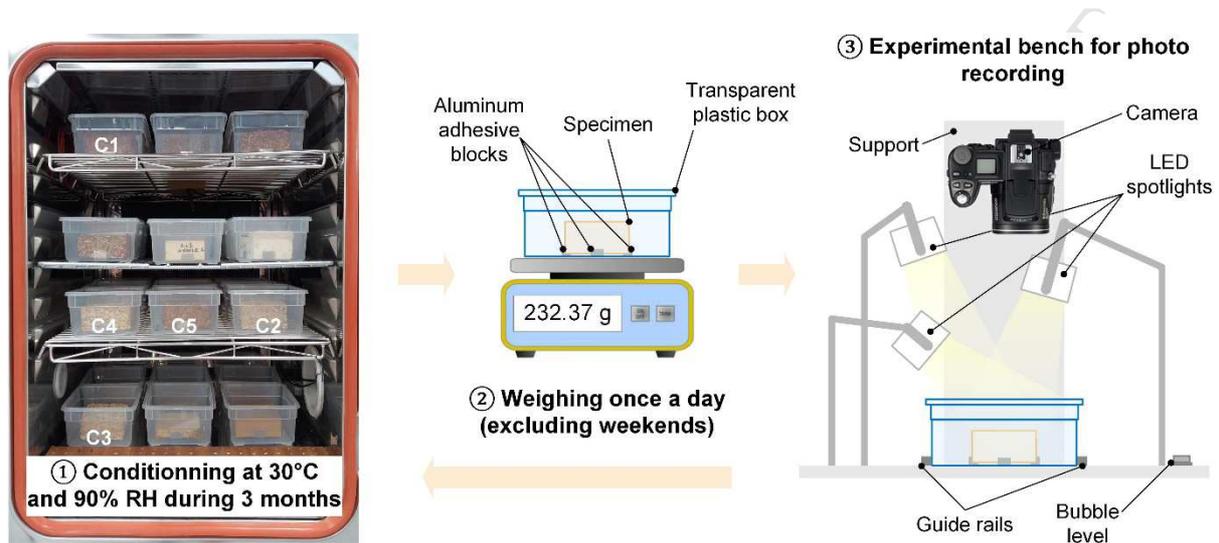
208

209 The chosen method has an advantage of being non-intrusive unlike other methods found in the
 210 literature:

- 211 • Use of adhesive to remove fungal spores from the substrate and then scanning of mold
 212 imprint [18];
- 213 • Scanning two sides of the specimen [19].

214 It should be noted that during the entire period of the test the biotope remains undisturbed.

215



216

217 *Figure 3: Experimental device for biological aging: conditioning, weighing and photo recording*

218

219 **2.4.2. Image analysis**

220 In this study, the chosen contamination indicator is the relative photographed surface contaminated by
 221 mold with reference to the total surface. A similar approach is used by various researchers in the field
 222 such as Nielsen et al [2], Johansson et al [10], Clausen and Yang [18] and Garzon-Barrero et al [19].
 223 To evaluate the mold growth between two dates t_0 and t , two photographs of the same specimen
 224 have been taken. In this study, a very simple experimental device has been chosen (Figure 3,
 225 Experimental bench for photo recording) to avoid use of sophisticated equipment. However for image
 226 analysis, it is necessary to use or to develop specific IT-tools.

227

228 The image analysis is performed in two steps:

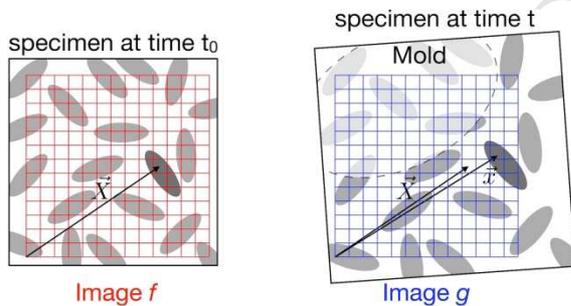
- 229 • Firstly, the displacement field between images recorded at t_0 and t is computed. This
 230 displacement may be due to operator handling (such as accidental motion of the specimen or
 231 the camera) or caused by a dilatation of the composite due to swelling induced by moisture
 232 change. This step is achieved, thanks to Digital Image Correlation (DIC) analysis, using the
 233 code DIGIMCO developed by Marc François in MATLAB software and freely available. Similar

234 tools, whether free or commercial software exist [20,21] and have been used for several years
 235 to perform displacement field measurement, or to evaluate mechanical properties [22–24].

236 • Secondly, the location of mold is revealed thanks to the error map of correlation between
 237 images recorded at time t_0 and t . It represents the discrepancy between the two images once
 238 correlated in space. A gray level difference reveals the presence of molds.

239

240 A digital image is a set of data produced by recording variation in the intensity of a signal. It's a matrix
 241 of r rows and c columns. Each pixel has a value that represents the brightness of a given color at any
 242 specific location. In this paper, the image f and the image g designate the images of an object
 243 recorded in the initial state (at time t_0) and in the final state (at time t) respectively. The discrete
 244 functions $f(\mathbf{X})$ and $g(\mathbf{X})$ denote the gray levels associated with pixel of the image f and g respectively,
 245 located at position \mathbf{X} (the coordinates are in pixels). The location of a given texture point is referred to
 246 be as \mathbf{X} in image f and \mathbf{x} in image g (see Figure 4). Possible transformations between two images are
 247 schematized in Figure 4 (i.e. dilatation, translation and rotation). The first step of the analysis is to
 248 compute the displacement field $\mathbf{u}(\mathbf{X})$ which gives the best fit between images f and g . The texture of
 249 the two images is quite similar and is used to determine this fit.



250

251 *Figure 4: Digital image correlation between initial image f (time t_0) and current image g (time t)*

252 The displacement \mathbf{u} is defined by:

$$253 \quad \mathbf{x} = \mathbf{X} + \mathbf{u} \quad (2)$$

254 Supposing a homogeneous deformation, the mapping of the transformation is a parameterized field of
 255 the form:

$$256 \quad \mathbf{x} = \mathbf{F} \cdot (\mathbf{X} + \mathbf{T}) \quad (3)$$

257 where \mathbf{T} is a translation vector described by the parameters λ_1 and λ_2 .

$$258 \quad \lambda_1 = T_1 \quad (4)$$

$$259 \quad \lambda_2 = T_2 \quad (5)$$

260 and \mathbf{F} is the deformation gradient tensor described by the parameters $(\lambda_3, \lambda_4, \lambda_5, \lambda_6)$:

$$261 \quad \lambda_3 = n (F_{11} - 1) \quad (6)$$

$$262 \quad \lambda_4 = n (F_{12}) \quad (7)$$

$$263 \quad \lambda_5 = n (F_{21}) \quad (8)$$

$$264 \quad \lambda_6 = n (F_{22} - 1) \quad (9)$$

265 where n is a weighting factor. It must be emphasized that the tensor \mathbf{F} includes pure deformation, rigid
 266 rotation and dilatation. The DIC method [22] is based on an assumption of optical flow conservation
 267 between images f and g whose texture does not significantly differ thus $f(\mathbf{X}) = g(\mathbf{x}) = g(\mathbf{X} + \mathbf{u})$ (with
 268 a possible error). The experimental conditions are compatible with these assumptions since the
 269 brightness of the images and the color of the composites do not significantly evolve during the test. To
 270 find the displacement \mathbf{u} or the parameters λ_i , the sum of squared differences φ of the gray levels have
 271 to be minimized over the zone of interest $-Z_i-$ which corresponds in this study to the whole
 272 photographed face of the composite is:

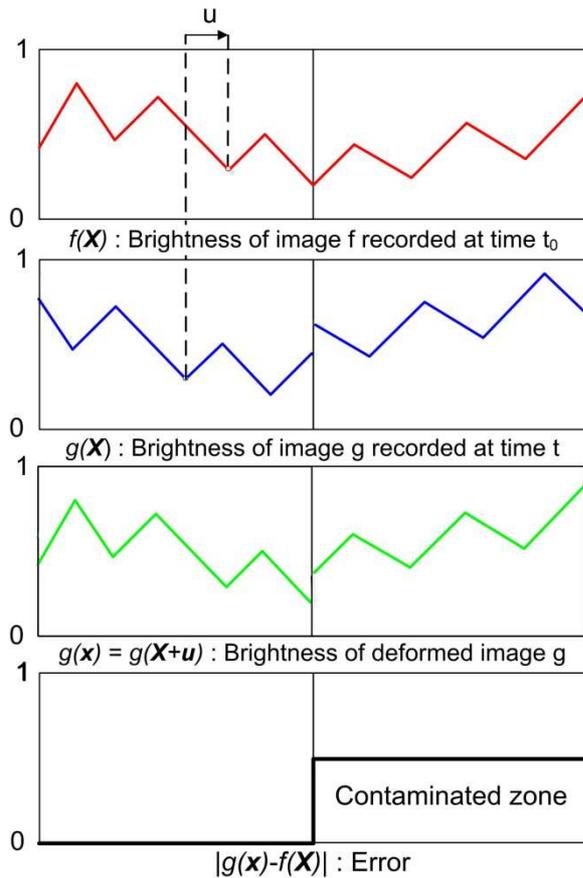
$$273 \quad \varphi(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5, \lambda_6) = \sum_{\mathbf{x} \in Z_i} (g(\mathbf{x}) - f(\mathbf{X}))^2 \quad (10)$$

274 However the computation of the displacement field \mathbf{u} is not the objective of this study. It is only
 275 necessary to compare images f (original) and g (with mold), pixel to pixel, at exactly the same location.
 276 Here, the presence of mold changes the gray level. The zones affected by mold are supposed to be
 277 large with respect to the texture size of the image of the composite. Then, assuming a uniform
 278 variation of the gray level b , (due to the presence of mold) the equation 10 becomes:

$$279 \quad \varphi(\lambda_1, \lambda_2, \lambda_3, \lambda_4, \lambda_5, \lambda_6) = \sum_{\mathbf{x} \in Z_i} (g(\mathbf{x}) + b - f(\mathbf{X}))^2 \quad (11)$$

280 The result of the minimization (i.e. the computation of parameters λ_i or the displacement field \mathbf{u}) is not
 281 modified by the presence of b since it does not modify the gradient of φ [22]. Figure 5 illustrates the
 282 method. The function $f(\mathbf{X})$ represents a 1D example of gray level in the initial image f (without mold).
 283 The function $g(\mathbf{X})$ represents the final grey level of image g in which molds have changed the gray
 284 level of some domains. A rigid body motion is visible. Once computed the best correlation (Equation
 285 11) the maximum and minimum shall match between $f(\mathbf{X})$ and $g(\mathbf{x})$. Then, the difference $g(\mathbf{x}) - f(\mathbf{X})$
 286 exhibits the location of the colonization.

287



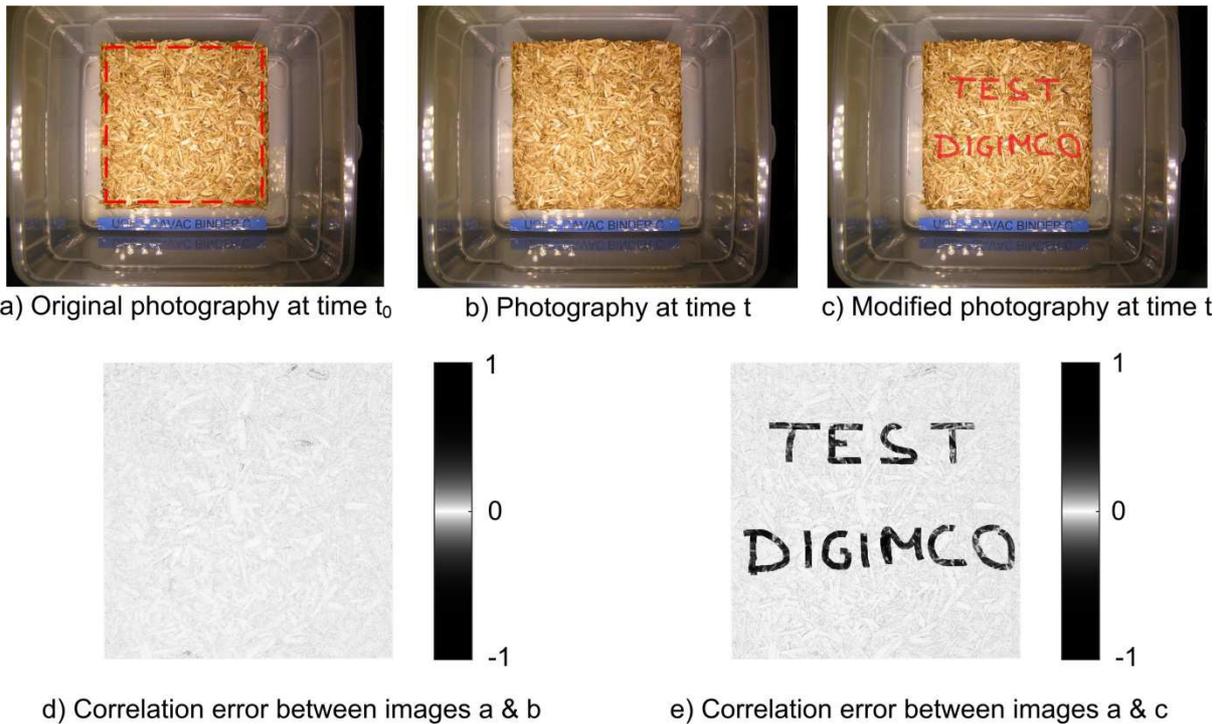
288

289 *Figure 5: Relative brightness, of image g and f : an error involves the detection of a new object.*

290

291 As an example, a test was performed on an 8-bit image (brightness coded from 0 to 256) of the
 292 composite (C1) without visible mold (see Figure 6). The reference image 6.a is compared with the
 293 image (6.b). It is a photography of the same composite taken 24 hours later also free of molds. In
 294 image 6.c an inscription is added with drawing software. Then, with DIGIMCO, the uniform
 295 displacement field which allows to superimpose the images 6.a and 6.b first, and then the images 6.a
 296 and 6.c are searched. Once the displacement field has been obtained, the map of the correlation
 297 errors between 6.a and 6.b is displayed on the image 6.d. On the image 6.e the map of the correlation
 298 errors between 6.a and 6.c is also displayed. On the error map 6.e, the inscription added to the image
 299 6.c is clearly visible while the error map 6.d is uniform and has virtually zero errors. From this error
 300 map, a thresholding operation (binarization) makes it possible to isolate the change, in this example,
 301 the inscription "Test DIGIMCO".

302



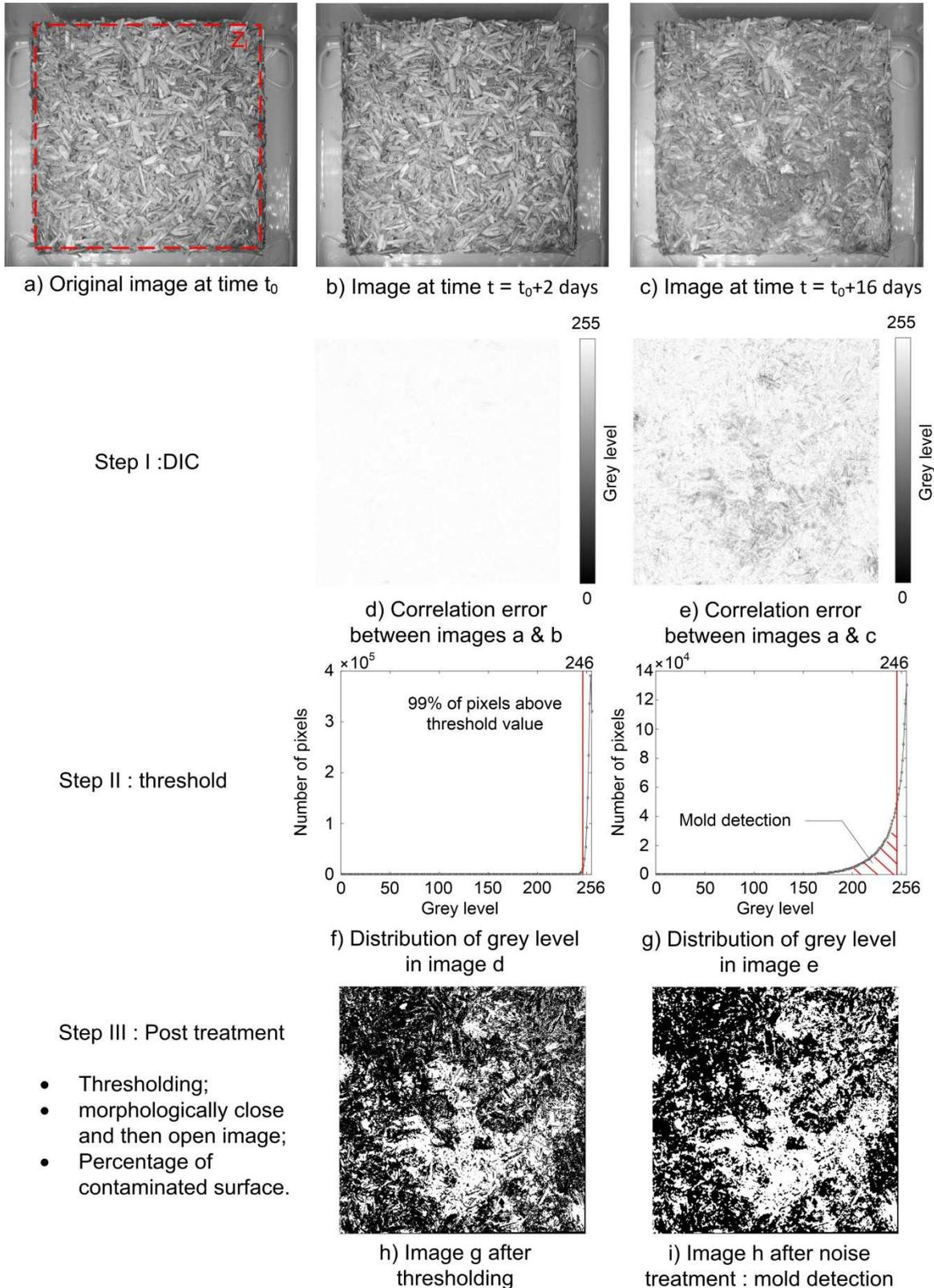
303

304 *Figure 6: Example of object detection using DIC*

305

306 It should be emphasized that in the traditional use of the DIC method, the surface of the studied object
 307 is covered with a randomly painted speckle pattern. This surface preparation allows to obtain a highly
 308 contrasted and unstructured image. This is not the case for this test. The surface of the composite acts
 309 as the randomly speckle pattern. The presence of hundreds of particles entangled without a regular
 310 pattern on the visible surface of the composite makes it possible to apply the DIC method without
 311 modifications. Thus, using the DIC does not require any treatment or modification of the environment
 312 likely to modify the growth of the studied organism.

313



314

315 *Figure 7: Procedure of image analysis, DIC and post-treatment applied on composite C5*

316

317 Once correlation error maps are calculated for each image, the post-processing operation is

318 performed with MATLAB. The entire image processing procedure is summarized in Figure 7. It

319 requires at least 3 pictures: the initial image of the composite 7.a, an image without apparent molds b)
320 and the image to be analyzed 7.c. After obtaining correlation maps 7.d and 7.e, a thresholding is
321 performed. The choice of the threshold value is by definition arbitrary. However, the study of the
322 correlation errors map obtained for an image supposed without fungi (see a sample at $t = t_0 + 2$ days
323 on Figure 7.d) allows to propose a coherent and systematic threshold value. On this example, 99 % of
324 the pixels have a gray level greater than 246 (see Figure 7.f). This value was used for the thresholding
325 operations performed on the photographs taken the following days.

326
327 Pixels which value is below this threshold value are considered to be contaminated (see Figure 7.g).
328 After the thresholding, the Figure 7.h is obtained). A morphological operation of opening and closing of
329 the black-and-white image is then performed to filter the isolated pixels (with the “imopen” and
330 “imclose” function of MATLAB) and reduce apparent measurement noise. Finally, the number of white
331 pixels and black pixels in the image are counted to get the percentage of surface contaminated by
332 fungi.

333

334 **2.4.3. Microscope visualizations**

335 After the three-month test period, microscope visualizations are performed in this study to identify the
336 type of mold which are developed on the contaminated composite surface. The infected shiv are
337 collected with a pair of tweezers and are observed under a microscope LCD (5 Megapixels, Bresser).
338 Pictures are captured at 50x and 125x magnification. Then, the pictures are opened with Image J
339 (NIH) with the addition of the Microscope Measurement Tools plugin (Fiji) to take measures and scale
340 bar is added. The microscope is calibrated for a 50x and a 125x magnification using an optical fiber to
341 $125 \pm 0.7 \mu\text{m}$ in diameter (Corning® SMF-28® Ultra Optical Fiber). The determined scale is the
342 average value of five measurements and is validated using a coated optical fiber to $242 \pm 5.0 \mu\text{m}$ in
343 diameter (Corning® SMF-28® Ultra Optical Fiber).

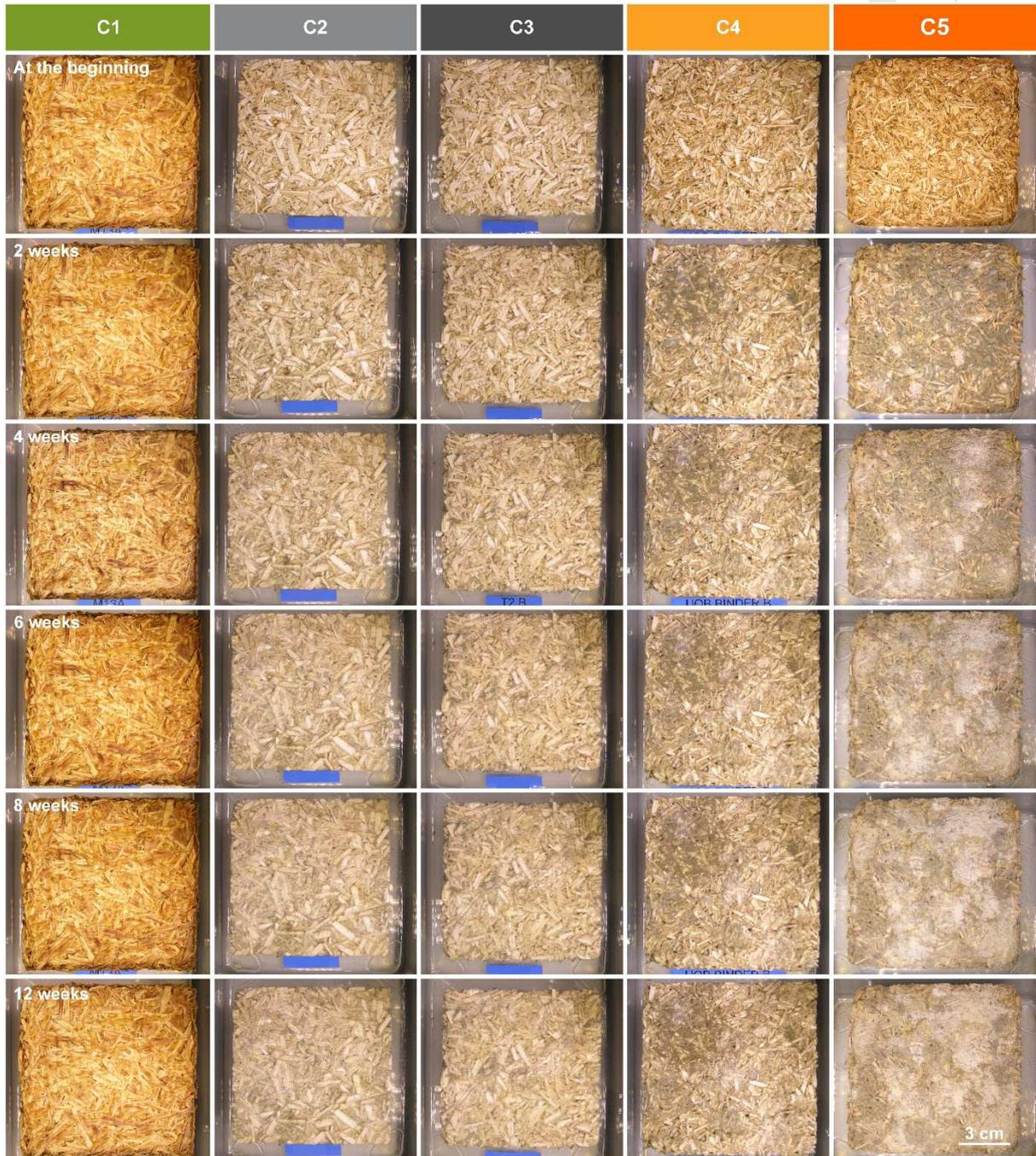
344

345 **3. Results and Discussions**

346 **3.1. Macroscopic visualizations**

347 Figure 8 gives the pictures of the C1 to C5 specimens at several times during the test period. The
348 visual inspection shows that during this test, C1 composite does not show any fungal development.

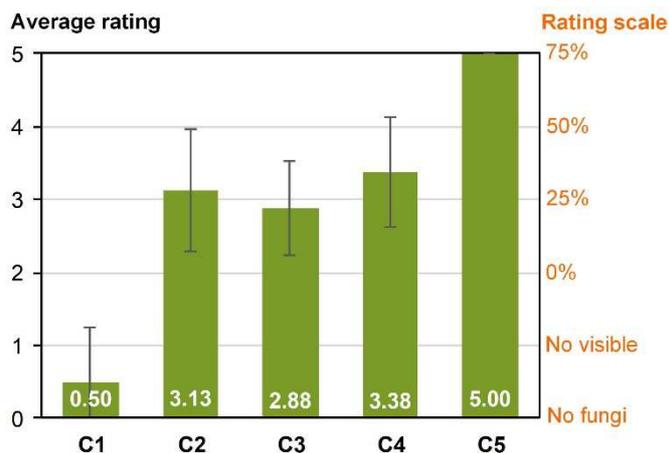
349 The other composites show gradual growth of molds, more or less, quickly. C2 and C3 composites do
 350 not have their surface strongly affected unlike the C4 and C5 composites. C5 composite has the most
 351 affected surface. First fungi mainly appear between the shiv on C2, C3, C4 and C5 composites
 352 between the fourth and the seventh day. The multiple colors of molds (grey, white and yellow)
 353 suggest at least two types of fungi have contaminated the composite surfaces.
 354



355
 356 *Figure 8: Specimens on the weeks 0, 2, 4, 6, 8 and 12 of ageing test*

357

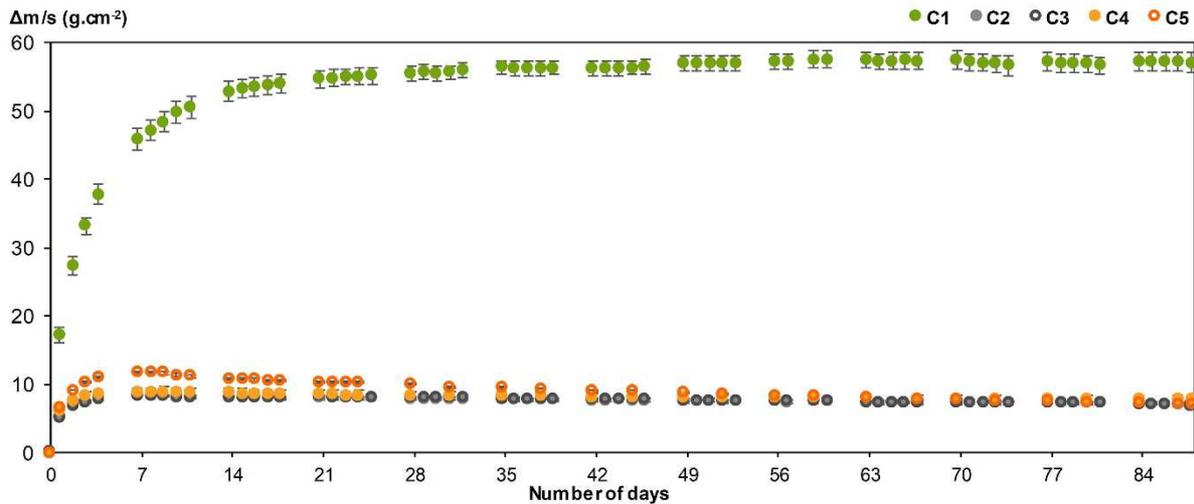
358 Figure 9 gives the average ratings of the visual evaluation given by the authors with the rating scale of
 359 BSI EN ISO 846 – 1997 [13] at the end of test. C1 composite does not have visible fungi. C3
 360 composite has less than 25 % of its surface contaminated. C2 and C4 composites have between 25
 361 and 50 % of their surface contaminated, with C4 being probably the more contaminated of the two.
 362 Finally, C5 composite has more than 75 % of its surface contaminated.
 363



364
 365 *Figure 9: Average rating of mold growth on specimens with the rating scale of BSI EN ISO 846 – 1997 (British*
 366 *Standards Institute, 1997) after 12 weeks at 30°C and 90% RH*

367
 368 **3.2. Mass loss**

369 Figure 10 gives the mass variation at 30°C and 90% RH from the steady state point (23°C and 50%
 370 RH), related to the exchange surface area (including top and lateral surfaces of specimens). During
 371 the first week of exposure, all specimens show an increase in mass due to water vapor adsorption
 372 resulting in a water content of about 20 % in specimens. From the eighth day, C5 composite slightly
 373 decreases in mass. From the ninth day, C2, C3 and C4 composites slightly decrease in mass too. The
 374 mass loss is due to the fungi development. C5 composite has the highest mass loss and C3
 375 composites has the lowest mass loss. The two others composites have similar mass loss. In contrast,
 376 from the eighteenth day, C1 formulation slowly increases in mass before stabilizing and reaching
 377 water content of about 54 % at the end of the test.
 378



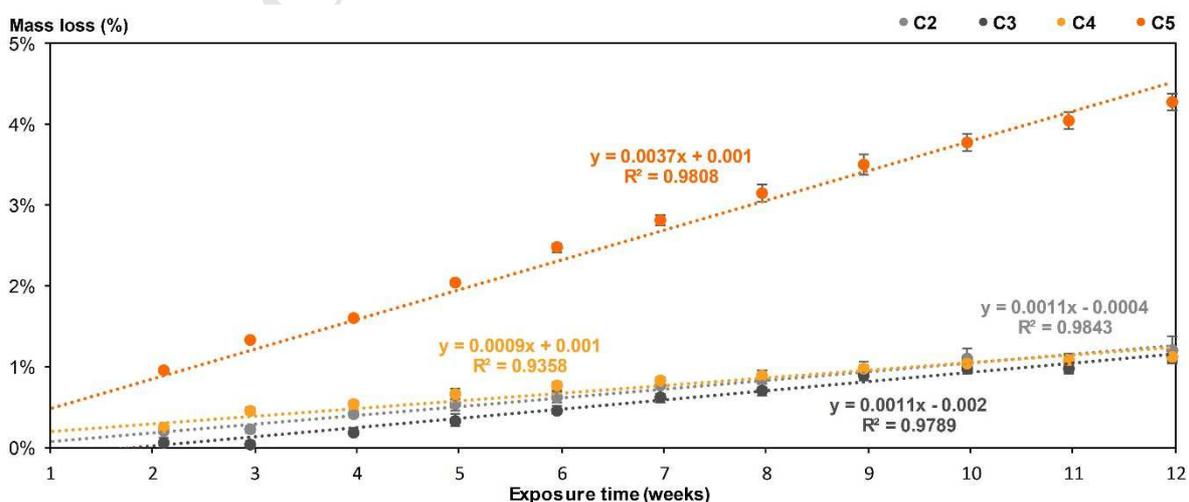
379

380 *Figure 10: Variation of mass (at 30°C, 90% RH) from the steady state point at (23°C, 50% RH), related to the*
 381 *exchange surface area*

382

383 Figure 11 and Table 5 give the mass loss for C2, C3, C4 and C5 composites during the test. The
 384 kinetics of mass loss increase linearly for the four formulations after the first week. This week certainly
 385 corresponds to the time needed to reach a water content threshold in the specimens in order to trigger
 386 mold growth. The correlation coefficients of the fitting curves are good as they are higher than 0.93.
 387 C5 composite has the highest mass loss. C3 composite has the lowest mass loss. This is probably
 388 due to the addition of paraffin which increases the water repellence. Moreover, the amount of
 389 adsorbed water by the C3 composite is lower than other composites. C2 and C4 composites have
 390 almost similar mass loss, thus the sol-gel coating does not have an impact on the kinetics of water
 391 vapor absorption. At the end of the test, these three formulations have a mass loss about 1.15 %
 392 whereas the C5 composite has the highest mass loss (4.28 %).

393



394

395 *Figure 11: Mass loss for the tested composites during the ageing test*

396

397 *Table 5: Mass loss (ML, %) for the tested composites according to the exposure time (weeks)*

Time (weeks)	ML C2	ML C3	ML C4	ML C5
0.96	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
2.11	0.21 ± 0.02%	0.06 ± 0.06%	0.23 ± 0.02%	0.94 ± 0.01%
2.96	0.23 ± 0.04%	0.03 ± 0.04%	0.45 ± 0.04%	1.33 ± 0.00%
3.96	0.42 ± 0.05%	0.19 ± 0.04%	0.53 ± 0.05%	1.61 ± 0.02%
4.96	0.53 ± 0.07%	0.33 ± 0.07%	0.66 ± 0.06%	2.03 ± 0.03%
5.96	0.62 ± 0.07%	0.46 ± 0.05%	0.76 ± 0.06%	2.47 ± 0.05%
6.96	0.76 ± 0.10%	0.62 ± 0.05%	0.82 ± 0.06%	2.81 ± 0.07%
7.96	0.85 ± 0.10%	0.71 ± 0.08%	0.89 ± 0.06%	3.15 ± 0.10%
8.96	0.95 ± 0.10%	0.90 ± 0.08%	0.97 ± 0.05%	3.50 ± 0.12%
9.96	1.10 ± 0.13%	0.97 ± 0.06%	1.04 ± 0.07%	3.78 ± 0.11%
10.96	1.05 ± 0.11%	0.98 ± 0.07%	1.08 ± 0.07%	4.04 ± 0.10%
11.96	1.21 ± 0.16%	1.13 ± 0.06%	1.12 ± 0.08%	4.28 ± 0.10%

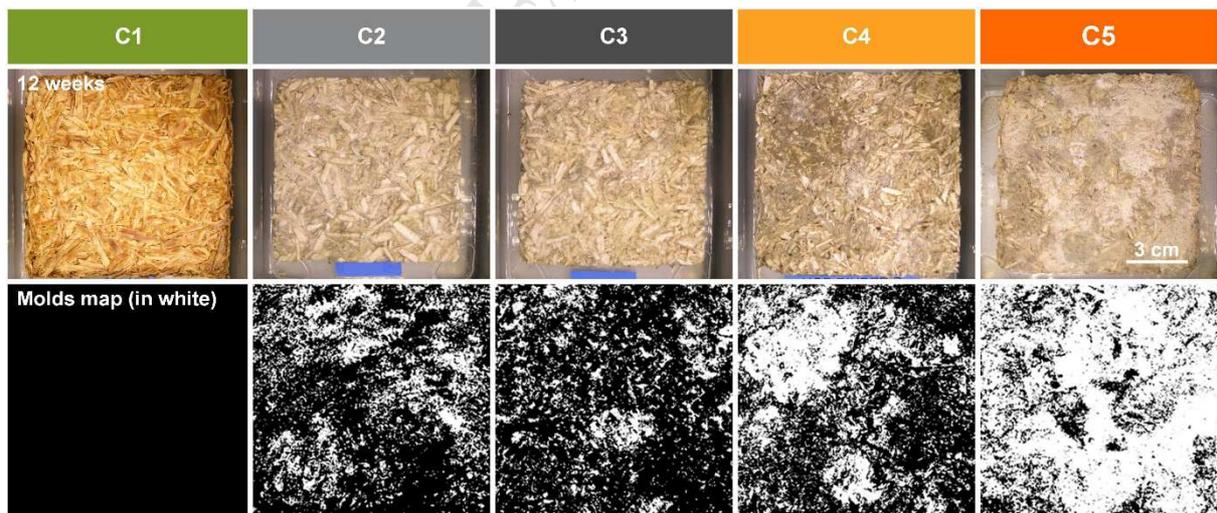
398

399

400 3.3. Image analysis

401 Figure 12 shows some pictures taken at the end of the test which are used to get the percentage of
402 surface contaminated by the fungi.

403



404

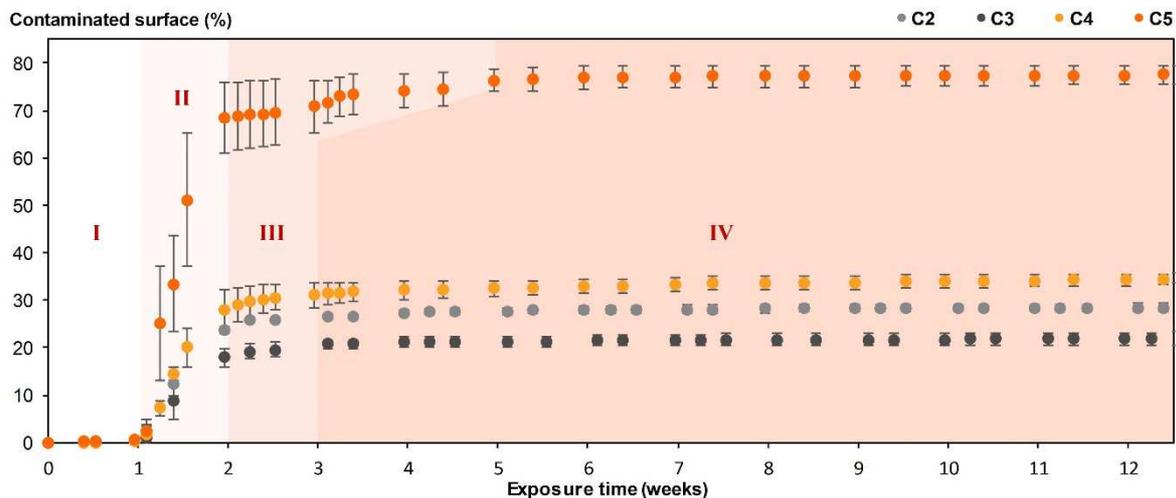
405 *Figure 12 : Pictures before and after DIC treatment at the end of the test*

406

407 The image analysis leads to the estimation of the surface ratio contaminated by the fungal growth
408 during the test period at 30 °C and 90% RH (Figure 13). The different mold growth rate between

409 formulations are observed. Overall, C5 specimens have greater fungal growth with a higher standard
 410 deviation between the three specimens. C3 specimens have the slowest rate of growth over the 12
 411 week test period. For all specimens, there is a substantial increase in growth during the second week
 412 which corresponds to the exponential phase of the fungal growth. During this period, the nutrients
 413 are in excess. Thus, the first week corresponds to the time required by the growth conditions to
 414 become favorable. The water content of specimens is around 10 % at the beginning of the test. 1
 415 week is required to double this value. This phase is called the lag phase. During the third week, the
 416 intensity of growth decreases. It corresponds to the slowdown phase. This is due to a depletion of the
 417 culture medium (composites) and an accumulation of waste. This phase goes on two weeks in the
 418 case of C5 composite. After the slowdown phase, the ratio of contaminated surface reaches a plateau
 419 until the end of the test. This means that the growth becomes zero and therefore the molds that grow
 420 multiply and replace those who die. This corresponds to the stationary maximum phase. At the end of
 421 test, the mean contaminated surface is 21.87 % for C3 specimens, 28.36 % for C2 specimens, 34.69
 422 % for C4 specimens and 77.50 % for C5 specimens. Growth kinetics observed here are consistent
 423 with what is described in the biology literature [25]. These results suggest that the image analysis
 424 method provides consistent and exploitable results for this study.

425



426

427 *Figure 13: Image analysis: rating of the contaminated surface of C2, C3, C4 and C5 composites exposed to 30°C*
 428 *and 90% RH during the test period correlated to the fungal growth phases with I: Lag phase, II: Exponential*
 429 *phase, III: Decelerating phase and IV: Stationary phase*

430

431 Table 6 compares the results obtained with the visual evaluation and the image analysis of the
 432 contaminated surface of specimens at the end of the test. The average rating from visual evaluation

433 and from image analysis are close. However, the visual evaluation shows high discrepancy (up to 0.83
 434 %) due to subjective effect. The image analysis leads to representative values as it is not subject to
 435 subjective effect. It seems to be more accurate.

436

437 *Table 6: Average rating from visual evaluation compared to average rating from image analysis of mold growth on*
 438 *specimens after 12 weeks at 30°C and 90% RH*

N°	C1	C2	C3	C4	C5
Visual evaluation	0.50 ± 0.76	3.13 ± 0.83	2.88 ± 0.64	3.38 ± 0.74	5.00 ± 0.00
Image analysis	0.00	3.13	2.93	3.43	5.00
Rating scale	No fungi	> 50 %	> 25 %	> 50 %	> 75 %

439

440 3.4. Microscopic visualizations

441 After the test period, a sampling of mold is collected in order to identify the type of fungi, which is
 442 responsible for the sample damage. Figure 14 shows some images of contaminated shiv taken using
 443 the optical microscope at different magnifications. Table 7 summarizes the fungi characteristics which
 444 have contaminated the specimens during the test. In the image 15.a, three species of mold are
 445 present. The one which has a long stem with fluffy spherical white head is the *Aspergillus ruber* (AR).
 446 It appears in more detail in the image 15.d. Under these white heads, a bright yellow spherical
 447 cleistothecium is hardly discernible. It is *Eurotium rubrum* (ER), the sexual form of *Aspergillus ruber*
 448 (AR) which is an asexual form. Still under these white heads, shiny smooth black globose pycnidia are
 449 slightly visible, which belong certainly to the *Phoma sp.* genus and could be of the *Leptosphaeria*
 450 *maculans* (LM) species. These two fungi appear in more detail in the image 15.e. In images 15.b and
 451 15.c, only one species is present. At the end of a long stem, there is a large white fan. It is the
 452 *Penicillium brevicompactum* (PB). In the images of the specimens (Figure 8), the colored areas in
 453 gray-brown correspond to this fungi. All contaminated composites (from C2 to C5) are infected by
 454 these four species of mold.

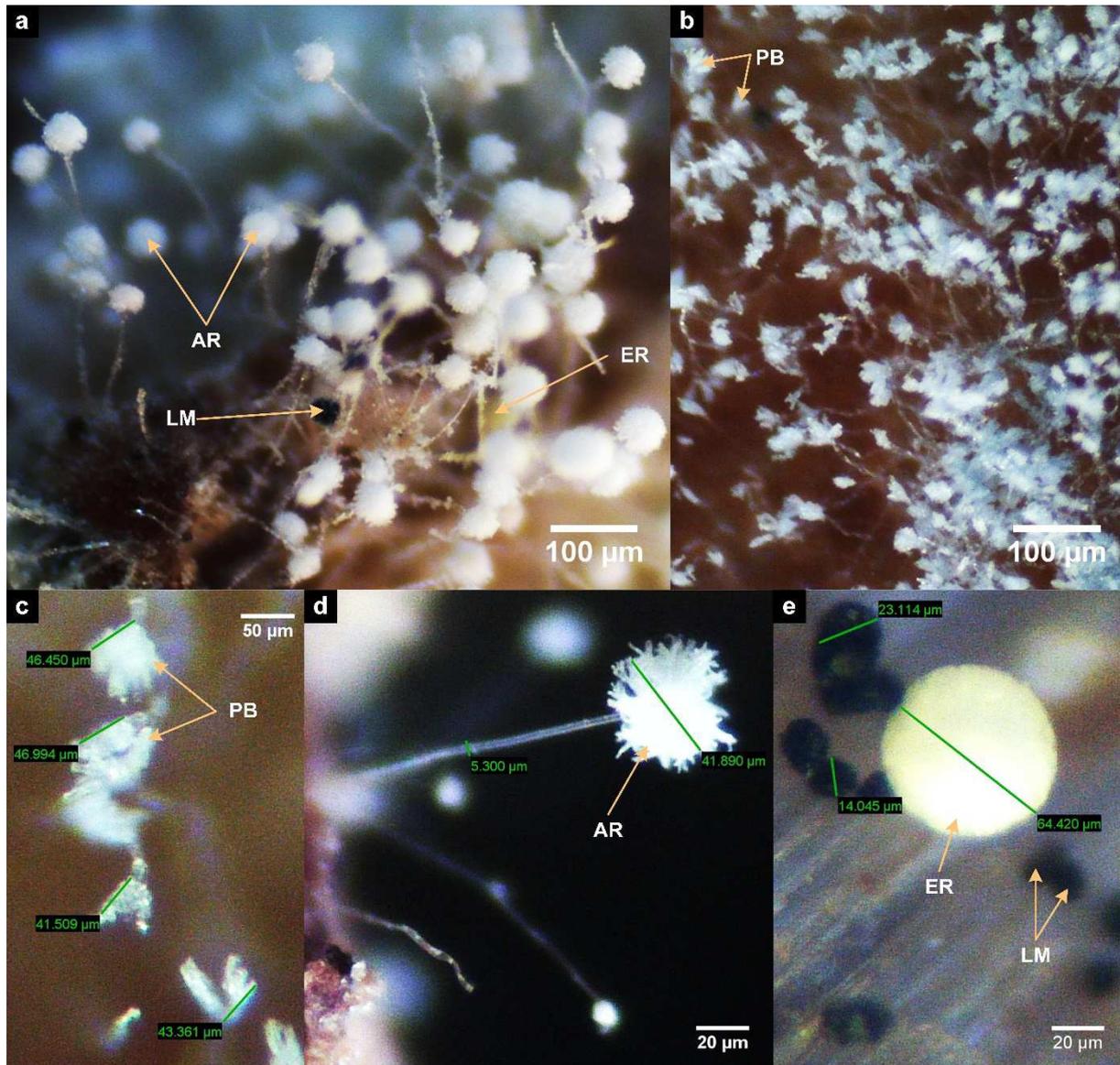
455

456 The fungi are divided in three types of colonizers which need a minimum of available water for fungal
 457 growth on the composites surfaces. The available water in the composites is defined by the water
 458 activity (a_w) which corresponds to the equilibrium relative humidity (ERH) in percent in the materials.
 459 Three colonizers are: primary ($a_w \leq 0.80$), secondary ($0.80 < a_w \leq 0.90$) and tertiary ($0.80 < a_w$) [26,27].
 460 *Penicillium brevicompactum* (PB), *Eurotium rubrum* (ER) and *Aspergillus ruber* (AR) are primary

461 colonizers whereas *Leptosphaeria maculans* (LM) is secondary colonizer and may gradually become
462 the dominant fungi. This means that the fungi belonging to the primary colonizers have appeared as
463 soon as the water activity in the specimens made it possible. The secondary colonizers has appeared
464 later, certainly towards the end of the test as these colonizers needs higher water activity than the
465 primary colonizers [28,29]. However, they may create more damage than the primary colonizers
466 because they prefer more complex sugars as cellulose for example [30]. Continuous mass monitoring
467 could have been interesting in order to verify this hypothesis.

468
469 Finally, all these molds are potentially allergens. Indeed, these molds are associated with allergic
470 reactions such as allergic rhinitis or worsening of asthma symptoms. The threshold of sensitivity varies
471 from one individual to another, and according to the type of mold for the same person. The individual
472 response and the experienced symptoms vary considerably depending on the individual sensitivity
473 and the duration of exposure to the fungi. Moreover, all molds contain inflammatory substances that
474 can produce inflammation to varying degrees.

475



476

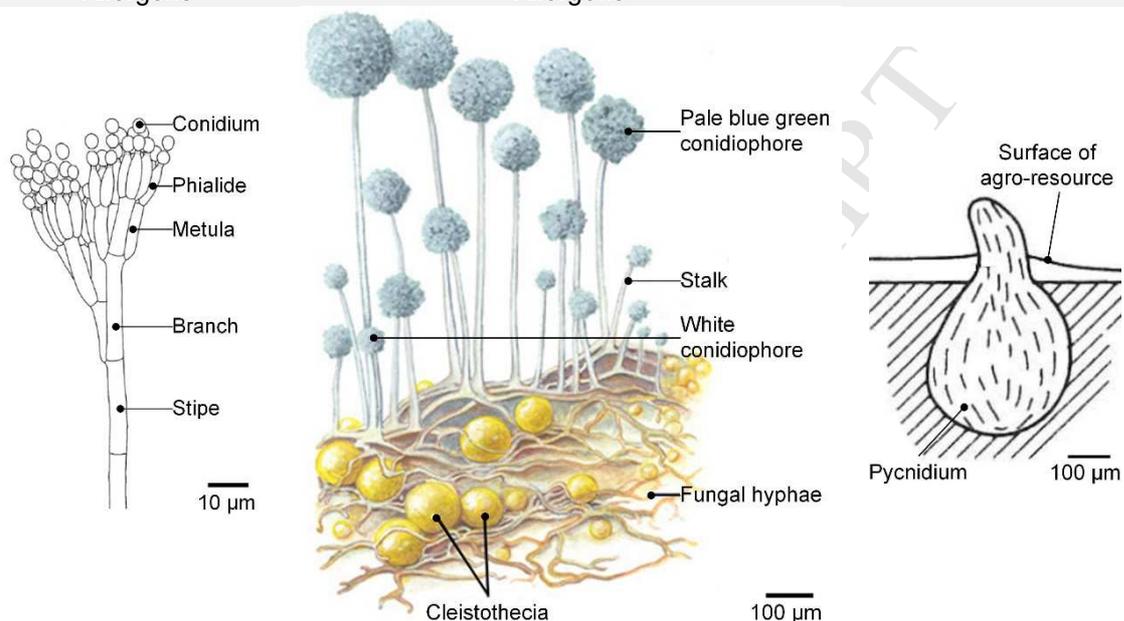
477 Figure 14: Optical microscope visualizations contaminated surfaces at the 50x (a, b and c) and 125x (d and e)
 478 magnifications (AR: *Aspergillus ruber*, ER: *Eurotium rubrum*, LM: *Leptosphaeria maculans* and PB: *Penicillium*
 479 *brevicompactum*)

480

481 Table 7: Fungi characteristics

Species	<i>Penicillium brevicompactum</i>	<i>Eurotium rubrum</i> (sexual form)	<i>Aspergillus ruber</i> (asexual form)	<i>Leptosphaeria maculans</i> (anamorph <i>Phoma lingam</i>)
Genus	<i>Penicillium</i> sp.	Trichocomaceae	<i>Aspergillus</i> sp.	<i>Phoma</i> sp.
Order	Moniliale	Eurotiales	Moniliale	Sphaeropsidale
Class	Hyphomycetes	Eurotiomycetes	Hyphomycetes	Coelomycetes
Phylum	Deuteromycota	Ascomycota	Deuteromycota	Deuteromycota
Growth	Can grow between -2 and 30 °C with an optimum at 23 °C	Can grow between 5 and 37 °C with an optimum at 25 °C		Can grow between 15 and 32 °C with an optimum between 20 and 24 °C
Microscopic Appearance	Stipes smooth walled about 500 to 800 μm	Bright yellow spherical cleistothecium about	White radiate conidial heads	Shiny smooth black globose pycnidium

	in length. Broad terverticillate penicillin less than 40 μm long and 40 to 50 μm across the phialide tips.	60 to 150 μm in diameter	about 30 to 50 μm in diameter. Stalk smooth until 750 μm in length and 5 to 6.5 μm in diameter.	until 500 μm in diameter and developing under the epidermis and tearing at maturity.
Toxicity	Mycophenolic acid: Allergens	Quinonoid pigments: Allergens		Allergens

Scheme**References**

[31–33]

[31,34,35]

[36–38]

482

483 4. Conclusions

484 A protocol for evaluating the resistance to mold contamination of bio-based composites is proposed in
 485 this paper. It could be used to assess decay resistance of composites before making them available
 486 on the market. Accelerated aging test involves placing the composites in a favorable environment to
 487 mold growth for 3 months (30°C; 90% RH). Resistance to colonization of composites is then evaluated
 488 over time using three tests:

- 489 ▪ Monitoring of external surface percentage contaminated by mold;
- 490 ▪ Mass monitoring;
- 491 ▪ Microscopic analysis.

492 The test protocol is easily reproducible since it requires commonly available equipment (a climate
 493 chamber, a camera and an optical microscope). The analysis protocol differs from existing intrusive
 494 fungal growth evaluation methods or based on subjective visual evaluations since it is fully
 495 automatized and do not needs any collecting of samples. The non-intrusive image analysis, based on
 496 the use of freely available IT-tools associated with mass monitoring allowed a rapid and quantitative
 497 assessment of mold growth and composite degradation.

498

499 Mass monitoring overlaps the effect of water absorption and decay. It provides information on long-
500 term degradation of composites. Moreover, it has been possible to determine the kinetics of apparent
501 mass loss. C1 composite does not show decay and the specimens increase in mass until they reach
502 equilibrium water content of about 54 % at the end of the test. For C2, C3, C4 and C5 composites, the
503 kinetics of mass loss increase linearly after the first week. This week certainly corresponds to the
504 needed time to obtain a sufficient water content in the specimens in order to trigger mold growth.

505

506 The percentage of the external surface contaminated by the composites is monitored by analyzing
507 images of the composites recorded during the test. The proposed method is based on an adaptation
508 of a DIC algorithm. Growth kinetics observed during the test is consistent with what is described in the
509 biology literature and suggests that the proposed method provides consistent and exploitable results.
510 The monitoring of the exterior surface contaminated by the composites allow a rapid and quantitative
511 evaluation of mold growth, essentially during the first 20 days.

512

513 The microscopic analysis make it possible to identify the species present on the composite. All
514 identified molds are potential allergens. These are mainly molds from primary colonizers. The
515 observation of a few individuals from secondary colonizers suggests that the degradation process of
516 the composites is still ongoing after 3 months of testing. Indeed, these colonizers would gradually
517 become the dominant fungi if nutrients are still available on the substrate. Moreover, they may create
518 more damage than the primary colonizers because they prefer more complex sugars. Continuous
519 mass monitoring could have been interesting in order to verify this hypothesis.

520

521 This test also makes it possible to establish a link between the chemical formulation of the composites
522 and their resistance to fungal colonization. The results suggest that the pH of given building material is
523 an important predictor of fungal susceptibility. Indeed, C1 formulation is the most resistant to fungal
524 development. This is due to having a surface pH of 10. The other formulations have a surface pH less
525 than or equal to 6 and have a more or less significant fungal development at the end of three months
526 of exposure test at 30°C and 90% RH. Another important predictor is the chemical composition of
527 specimens. However, C3 formulation contains paraffin to increase the water repellence. Thus, the

528 water content in the C3 specimens is lower than other specimens leading to lower kinetics of mold
529 growth on its exposed surface.

530

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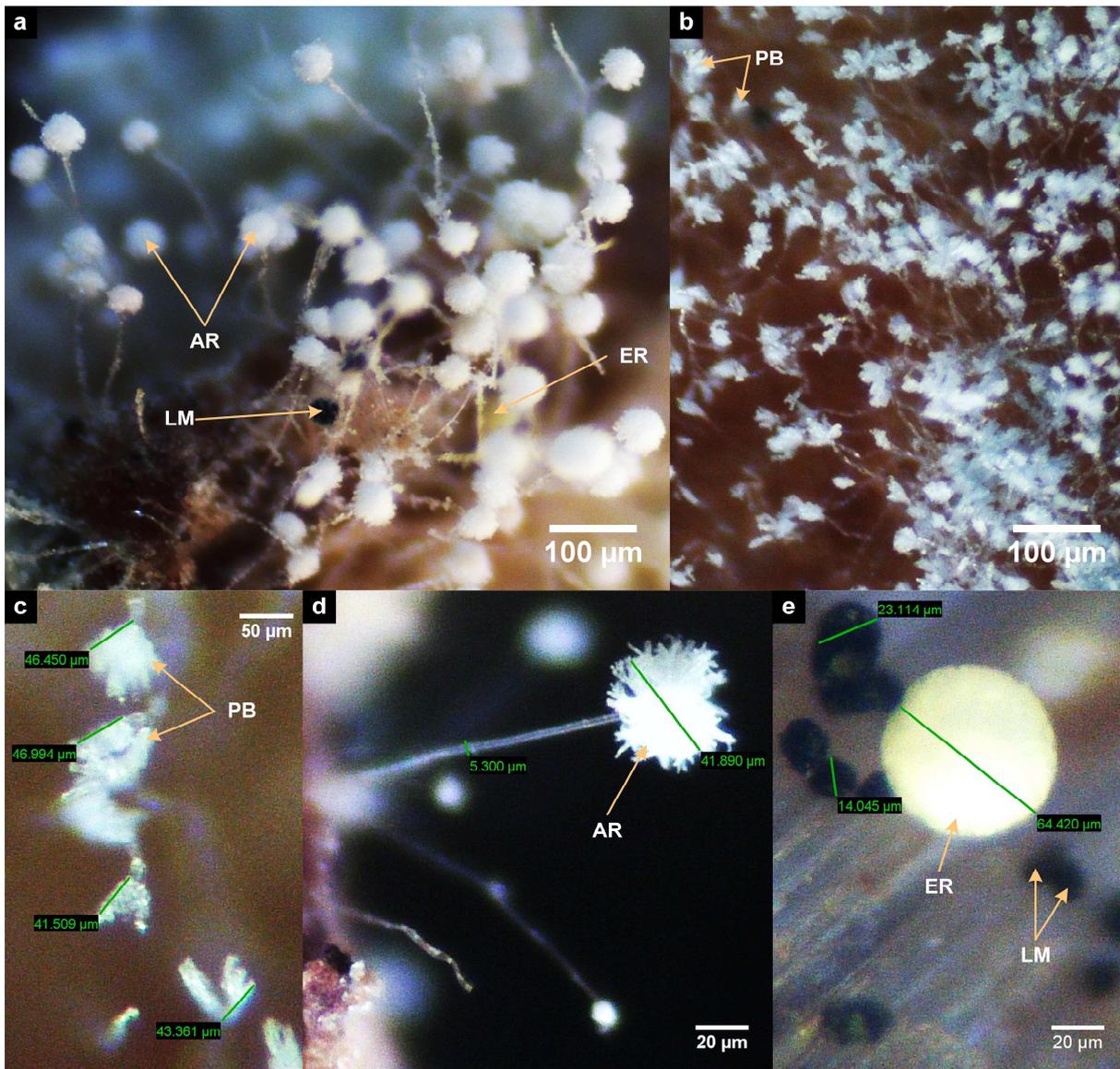
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- 625

- 1 *Figure 1 : Flow-chart of composites production (except C4 formulation)*
- 2 *Figure 2 : Developed composites*
- 3 *Figure 3: Experimental device for biological aging: conditioning, weighing and photo recording*
- 4 *Figure 4: Digital image correlation between initial image f (time t_0) and current image g (time t)*
- 5 *Figure 5: Relative brightness, of image g and f: an error involves the detection of a new object.*
- 6 *Figure 6: Example of object detection using DIC*
- 7 *Figure 7: Procedure of image analysis, DIC and post-treatment applied on composite C5*
- 8 *Figure 8: Specimens on the weeks 0, 2, 4, 6, 8 and 12 of ageing test*
- 9 *Figure 9: Average rating of mold growth on specimens with the rating scale of BSI EN ISO 846 – 1997 (British*
10 *Standards Institute, 1997) after 12 weeks at 30°C and 90% RH*
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12 *exchange surface area*
- 13 *Figure 11: Mass loss for the tested composites during the ageing test*
- 14 *Figure 12 : Pictures before and after DIC treatment at the end of the test*
- 15 *Figure 13: Image analysis: rating of the contaminated surface of C2, C3, C4 and C5 composites exposed to 30°C*
16 *and 90% RH during the test period correlated to the fungal growth phases with I: Lag phase, II: Exponential*
17 *phase, III: Decelerating phase and IV: Stationary phase*
- 18 *Figure 14: Optical microscope visualizations contaminated surfaces at the 50x (a, b and c) and 125x (d and e)*
19 *magnifications (AR: Aspergillus ruber, ER: Eurotium rubrum, LM: Leptosphaeria maculans and PB: Penicillium*
20 *brevicompactum)*



RESEARCH HIGHLIGHTS

- New rapid method for laboratory scale testing of materials resistance to molds.
- The fungal resistance of new bio-based buildings materials is analyzed.
- Mass monitoring overlaps the effect of water absorption and decay.
- DIC is used to analyze images. Correlation error indicates presence of mold.
- The pH of given building material is an important predictor of fungal susceptibility.