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1 **Avrami's law based kinetic modeling of colonization of mortar surface by alga**  
2 ***Klebsormidium flaccidum***

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4  
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16  
17  
18  
19 **Nomenclature**

20 X(t) colonization rate (%)  
21 t time (day)  
22  $t_l$  latency time (day)  
23  $\gamma$  number of algal spots at time t per unit area  
24  $dy/dt$  specific attachment rate (Spot/ $\mu\text{m}^2$ .day)  
25 S surface of an algal spot ( $\mu\text{m}^2$ )  
26  $k_g$  specific attachment constant (Spot/ $\mu\text{m}^2$ .day<sup>2</sup>)  
27  $k_c$  growth rate constant ( $\mu\text{m}/\text{day}$ )  
28 K overall rate constant (Spot/day<sup>4</sup>)  
29 n Avrami's exponent

30  
31 **Abstract**

32 The aim of this research was to modelize the colonization of mortar surface by green algae  
33 using Avrami's law. The resistance of mortars, with different intrinsic characteristics  
34 (porosity, roughness, carbonation state), to the biofouling was studied by means of an  
35 accelerated lab-scale test. A suspension of green alga *Klebsormidium flaccidum*, was  
36 performed to periodically sprinkle the mortar surfaces. The covered surface rate followed a  
37 sigmoidal type curve versus time. Moreover, in order to apply Avrami's law, the algal  
38 colonization has been described by two processes: attachment and growth of algal cells. The  
39 image analysis showed that both the roughness and the carbonation influenced the algal  
40 attachment, unlike the porosity. The attachment specific rate increased linearly with time. For  
41 the algal growth process, it's difficult to conclude on the influence of mortar characteristics  
42 due to a high dispersion of experimental results. However, the assumption of a constant  
43 growth rate was acceptable. A good agreement between the simulation and the experimental  
44 results was obtained.

45  
46 **Keywords:** modeling, Avrami's law, biological colonization, algae, mortar

47  
48 **1. Introduction**  
49

50 The building facades, after construction, are inevitably subject to the colonization by  
51 microorganisms which can induce an aesthetical degradation of the construction. These  
52 microorganisms may be bacteria, algae, cyanobacteria, fungi, lichens and even higher plants if  
53 no prevention is achieved. It is found that, except ubiquitous bacteria, algae are the first  
54 colonizers. Moreover, the *Klebsormidium flaccidum* algae are well known for their wide  
55 distribution (John 1988 ; Ortega-Calvo et al. 1991 ; Ortega-Calvo et al. 1993 ; Gaylarde and  
56 Gaylarde 2000 ; 2005 ; Crispim et al. 2003 ; Rindi et al. 2008) and like being the dominant  
57 microorganism in biofilm composition (Barberousse et al. 2006). The biological biofouling  
58 causes an aesthetic problem and thus a significant economic loss due to the maintenance and  
59 the repair of the facades.

60 The implantation of microorganisms depends on various-origins parameters. Indeed,  
61 under temperate or tropical climate, the composition of a biofilm and the dominant species are  
62 respectively different (Crispim et al. 2003). According to several authors the microclimate  
63 (i.e. light, moisture) is an essential parameter which controls the nature and the growth of the  
64 microorganisms (John 1988 ; Ariño et al. 1997). The microclimate is influenced by the  
65 inclination, the orientation, the distance from ground and the exposure to the shadow of a  
66 building facade. The substrate characteristics, such as porosity, surface roughness, chemical  
67 composition and surface pH, are obviously important (Deruelle 1991 ; Ortega-Calvo et al.  
68 1995 ; Tomaselli et al. 2000 ; Tran et al 2012). Several authors showed that the biological  
69 colonization is faster on a rough wall (Wee and Lee 1980 ; Pietrini et al. 1985 ; Joshi and  
70 Mukudan 1997 ; Tomaselli et al. 2000 ; Tran et al. 2012). According to these authors, the  
71 roughness promotes the attachment of biological cells by providing numerous asperities.  
72 Moreover, a high porosity increases the amount of water available to microorganisms and  
73 thus favors their development (Ohshima 1999 ; Prieto and Silva 2005 ; Miller et al. 2006 ;  
74 Miller et al. 2009). On the contrary, the settlement by algae is slowed down or inhibited if the  
75 surface pH is greater than 11 (Grant 1982).

76 Several studies focused on the influence of these parameters on biofouling, at laboratory  
77 scale as well as at real scale. However, to our knowledge, very few studies have attempted to  
78 model this phenomenon. Ruot and Barberousse (2007) proposed a simulation of the surface  
79 materials colonization by algae. They showed that the Avrami's equation was quite a good  
80 tool to express the temporal evolution of the colonization rates. However, their study remains  
81 still preliminary and does not allow explaining the kinetic process and the role of material  
82 intrinsic characteristics.

83 Avrami's law, in the form of an exponential equation, has been developed by Avrami,  
84 Johnson and Melh for over 70 years (Avrami 1939 ; 1940 ; 1941 ; Johnson and Mehl 1939).  
85 This law was originally intended to describe the allotropic phase transformation in solids.  
86 Nowadays, it is used in many domains: crystallization of polymers, heat treatment in industry  
87 and thermal decomposition of solids ... (Hay 1971; Slováček 2004). Avrami's model is based  
88 on two processes: the nucleation and the growth. The nucleation corresponds to the  
89 appearance of nuclei of a new phase. The growth represents the increase in the size of these  
90 nuclei into the initial phase during time.

91 In the case of this work, the fouling is initiated by the attachment of algae on the surface  
92 of the samples which creates many spots. The colonization rate follows a sigmoidal curve for  
93 the surface fraction colonized as function of time. As a consequence, colonization can be  
94 simulated thanks to the Avrami's model considering the algal spots as nuclei. Indeed, the  
95 algal spots are very small and randomly distributed on the surface. The extension of the  
96 fouling results from the increase in the size of the first algal spots by the growth of algal  
97 filament and from the adhesion of new spots as a function of time.

98

99 The aim of this study is to modelize the colonization mechanism of the surface materials  
100 by algae using Avrami's model.

## 103 2. Materials and methods

104  
105 The studied materials were mortars made up of Portland cement CEM I 52.5 N (Holcim),  
106 siliceous sand (Sibelco DU 0.1/0.35) and calcareous filler (Omya). Table 1 gives the  
107 proportions of each constituent. The mortar was prepared with a water to cement ratio w/c  
108 (wt./wt.) of 0.5. In order to obtain a more porous mortar, the w/c ratio was increased to 1.  
109 However, cellulose ether (Hydroxyethyl Methyl Cellulose - HEMC) was added as admixture  
110 to thicken the mortar and thus avoiding segregation.

111  
112 For each mortar, three finishing methods were applied on the surface of samples during  
113 the setting. One corresponded to a smoothing by a ruler and the two other to a scratching by  
114 sponges of two different roughnesses.

115  
116 The mortar mixture was cast into  $50 \times 50 \times 1$  cm expanded polystyrene moulds and  
117 stored at  $21 \pm 1$  °C and  $95 \pm 5$  % of relative humidity (RH) during 28 days (for the  
118 preparation of uncarbonated mortars). To prepare carbonated samples, the mortars were stored  
119 only 7 days before being cut into  $20 \times 8 \times 1$  cm samples. These samples were then stored in a  
120 chamber under pure CO<sub>2</sub>, at  $21 \pm 1$  °C, and  $65 \pm 5$  % relative humidity during 36 days.

121  
122 The total porosity of materials was determined by mercury intrusion porosimetry  
123 (Micromeritics Autopore IV 9400). For each mortar, three samples beforehand dried by  
124 acetone were analyzed.

125  
126 The surface roughness was measured using a CHR-150-L profilometer and was evaluated  
127 by the arithmetic average of the height ( $R_a$ ) (Gadelmawla et al. 2002).

128 The surface pH of mortars was measured by a surface electrode (WTW Sentix Sur). To  
129 ensure contact between the substrate and the pH-electrode, a drop of water was deposited on  
130 the surface before measurement.

131  
132 The studied algal specie was *Klebsormidium flaccidum*. It was chosen due to its  
133 representativeness and its facility of cultivation.

134  
135 The bio-receptivity of mortar, depending on its characteristics (porosity, roughness,  
136 carbonation state), was examined through a laboratory accelerated test. The experimental  
137 device consisted of a  $100 \times 50 \times 50$  cm closed glass chamber placed in a dark room. At the  
138 beginning of the experiments, 50 L of sterilized Bold's Basal Medium (BBM) (Barberousse  
139 2006) were inoculated with *K. flaccidum* in order to obtain an algal suspension of  $4 \text{ mg.L}^{-1}$  of  
140 dry mass. The algal growth was carried out at 24 °C by means of a thermo-regulator. In this  
141 device, two rows of samples were placed back to back on a stainless steel support inclined at  
142 45°. Each row of samples was equipped with a system constituted of a stainless steel tube (10  
143 mm diameter drilled every 10 mm) and two pumps (Rena Flow 650 BF). This device allowed  
144 to the algal suspension, to flow on the top of each sample.

145 The sprinkling period and the flow rate were set respectively at 90 min every 12 h and at  
146  $26 \pm 2 \text{ L.h}^{-1}$ . The light was provided by two neon lamps (OSRAM Fluora L30W/77). The  
147 photoperiod was of 12 h and was starting with the beginning of a sprinkling cycle.

148

149 In each test, 18 samples of materials were placed into the chamber. Each formulation was  
150 tested in triplicates. Carbonated or uncarbonated samples were tested separately.

151 Table 2 gives the characteristics of the studied mortars. Each mortar was labeled  
152 according to three codes. The first one was the w/c ratio (0.5 or 1), the second one expressed  
153 the carbonation state (C for carbonated and UC for uncarbonated) and the third one  
154 corresponded to the roughness (R1, R2 and R3). For example, a sample labeled 10C-R2  
155 corresponded to a carbonated sample, carried out with a w/c ratio equal to 1 and with the  
156 intermediate roughness.

157 To evaluate biofouling, the sample surface was daily digitized by means of an office  
158 scanner. The area colonized by algae was determined by image analysis using Aphelion<sup>®</sup>  
159 software. The colonization rate was given by the ratio of the colonized area to the total surface  
160 and was noted X(t).

161 Full details of the experimental approach were described in a previous paper (Tran et al.  
162 2012).

163 In order to experimentally determine the specific attachment rate and the growth rate of  
164 algae, an algorithm was developed with Aphelion<sup>®</sup> software. The objective of the algorithm  
165 was to quantify new spots appearing between time t and t+ $\Delta t$  by comparison of the two  
166 images acquired respectively at time t and t+ $\Delta t$ . The growth rate was determined by following  
167 the increase in the surface of each spot over time.

168  
169

### 170 3. Theoretical basis of the model

171

172 An example of biodeterioration result is shown on Fig. 1a. On this figure, the effect of the  
173 roughness on the biofouling of mortars by algae is highlighted for the mortar serie 10C (10  
174 and C mean a w/c equal to 1 and carbonated, respectively). In fact, whatever the mortars  
175 studied, the shape of the curve, X(t), was always the same: sigmoidal type (Tran et al. 2012).  
176 In all cases, three steps were identified: a latency step, an exponential growth step and a step  
177 of stagnation (Tran et al. 2012). This type of curve can be well simulated by using Avrami's  
178 equation (Ruot and Barberousse 2007). As mentioned in the introduction, the Avrami's law is  
179 used to modelize the kinetics of allotropic phase transformations in solids. This model is  
180 based on two processes: the nucleation and the growth of nuclei. The nucleation corresponds  
181 to the formation of nuclei of a new phase. The growth corresponds to the increase in the size  
182 of these nuclei in the initial phase during time. However, the Avrami's law is only valid under  
183 the four assumptions below:

- 184 – the volume of the initial phase is unlimited compared to the nucleus one,
- 185 – the nuclei must be distributed randomly in the volume of the solid,
- 186 – the form and the growth of all the nuclei are identical,
- 187 – the growth rate is independent of the appearance time of the nucleus.

188

189 According to our biofouling results, an analogy between a chemical transformation and  
190 the fouling can be drawn. Thus it allows applying Avrami's theory. Indeed, during an  
191 allotropic transformation, the old phase is transformed into new one. In the case of the  
192 biological fouling of mortars, it is possible to consider the uncolonized mortar as the old  
193 phase and the mortar colonized by algae (algae attached on the surface of the mortar) as the  
194 new phase. In both cases, the transformation of the old phase into new one results from the  
195 displacement of the interface between both phases. Moreover, if we consider the algal  
196 biofouling of mortars, the curves  $dX/dt=f(X)$ , such as those in Fig. 1b, exhibit a maximum. In  
197 the case of chemical reaction, such as thermal decomposition of solid, it suggests that the  
198 reaction involves simultaneous nucleation and growth processes of the new phase (Galwey

199 and Brown). Kinetics modeling of these reactions are usually done using a general expression  
200 of the reaction rate under the form  $dX/dt=A.f(X)$ . A is a preexponential factor, which depends  
201 on temperature and activation energy and  $f(X)$  a function of the fractional conversion X.  
202 When  $X(t)$  is a sigmoidal curve, authors often use the Avrami laws to express  $f(X)$ . As it is  
203 shown on fig. 1a, in our case, the colonization rate  $X(t)$  corresponds to a sigmoidal curve.

204 Moreover, the sample fouling is initiated by the attachment of algae on the surface of the  
205 samples. It results in the appearance of very small green spots on the surface, corresponding  
206 to algae, which can be assimilated to nuclei in the Avrami's model. The smaller algal spot  
207 detected by image analysis, i.e. one pixel, was about  $7200 \mu\text{m}^2$ . This surface is very small  
208 compared to the samples size ( $160 \cdot 10^8 \mu\text{m}^2$ ). Thus the sample may be considered as infinite,  
209 as required by a two-dimensional Avrami's model. The thickness of algae biofilm was not  
210 investigated here.

211 The extension of the fouling results from the growth of the first algal spots and from the  
212 adhesion of new ones (Fig. 2). This confirms that the attachment and the growth occurred  
213 simultaneously, which allows the use of Avrami's model.

214 In addition, the algal spots appeared randomly on the sample surface as required by the  
215 Avrami's model.

216 Thus, the colonization by algae has been considered as the combination of two processes:  
217 the attachment and growth of algal spots, which were assimilated respectively to the  
218 "nucleation" step and to the growth step of the "nuclei" described in the Avrami's model.

219  
220 To simplify the modeling, it was assumed that the attachment ("nucleation" in the  
221 Avrami's model) occurred with the same probability over the entire surface of the sample. As  
222 a consequence, the surface defects, such as air bubbles, did not affect the attachment of algae.

223 Fig. 1 showed that no colonization of the sample surfaces by algae occurred during  
224 several days. This time was called latency period. During this period the attachment and the  
225 growth of algae were null. The duration of this phase can depend on several parameters, such  
226 as sprinkling period, chemical composition of the specimen and of the culture medium... The  
227 latency time ( $t_l$ ) was defined as the time corresponding to a colonization rate of 0.5 %. The  
228 best reproducibility was obtained using this method.

229 In general, the attachment and the growth of algae on the mortar surface might be  
230 affected by the porosity ( $p$ ), the roughness ( $R$ ), the initial pH of the surface ( $pH$ ), the chemical  
231 composition of the mortar, the algae species, the algae viability, the light intensity, the  
232 temperature, the inclination of the substrate surface and the sprinkling flow. However, except  
233 the material properties (porosity, roughness and surface pH), all other experimental  
234 parameters were constantly fixed.

235  
236 The specific attachment rate was defined as the number of algal spots appearing on  
237 surface unit per time unit (eq.1).

238  
239 
$$\frac{d\gamma}{dt} = k_g (p, R, pH) \times (t - t_l)^q \quad (1)$$

240  
241 Where:

242  $\gamma$  is the number of algal spots at time  $t$  per unit area ( $\text{Spot}/\mu\text{m}^2$ ).

243  $k_g$  is the attachment specific rate constant ( $\text{Spot}/\mu\text{m}^2 \cdot \text{day}^{q+1}$ ).

244  $t_l$  corresponds to the latency time (day).

245

246 Concerning the growth, the hypotheses that the growth rate was identical for all algal  
 247 spots and was constant during time were applied. The surface area covered by an algal spot  
 248 appearing at time  $\theta$  was expressed as follow:  
 249

$$250 \quad s_g(t, \theta) = k_c^2 (t - \theta)^2 \quad (2)$$

251  
 252 Where  $k_c$  corresponds to the specific growth rate constant ( $\mu\text{m}/\text{day}$ ).  
 253

254  $k_c$  takes into account the growth rate in each direction (x and y) and the form of the algal  
 255 spot.

256 The colonization rate  $X(t)$  was calculated from the law of "nucleation" and growth  
 257 previously expressed, as an exponential equation (eq. 3) (Delmon 1969).  
 258

$$259 \quad X(t) = 1 - e^{-K(t-t_1)^n} \quad (3)$$

260  
 261 Where:

$$262 \quad K = A k_g k_c^2 \quad (4)$$

$$263 \quad \text{with } A = \frac{2}{(q+1)(q+2)(q+3)} \quad (5)$$

$$264 \quad n = q + 3 \quad (6)$$

## 265 266 **4. Results**

### 267 268 **4.1. Experimental examination of algal attachment process**

269  
 270 The determination of the specific attachment rate of algae was carried out by the  
 271 algorithm developed with Aphelion software. At each time step, new algal spots fixed on the  
 272 surface were counted by image analysis. The specific attachment rate was thus calculated for  
 273 each material by dividing the number of new algal spots per unit of time by the initial sample  
 274 surface. The size of the smallest algal spot which could be detected was approximately  $85 \times$   
 275  $85 \mu\text{m}$  ( $7200 \mu\text{m}^2$ ). It corresponded to around 80 times the surface of an algal cell but only  $4.5$   
 276  $10^{-7}$  times the sample surface. This justified the hypothesis that the surface of the sample  
 277 could be considered as infinite as required by the Avrami's model.

278 This process was applied only in the early stage where less than 8 % of sample surface  
 279 was covered by algae. A more advanced colonization disrupted the fixation of new algal cells  
 280 and therefore distorted the determination of the specific attachment rate constant ( $k_g$ ).  
 281

#### 282 4.1.1 Influence of mortar characteristics on the algal attachment process

283  
 284 Fig. 3 illustrates the evolution, during time, of the specific attachment rate of algae for  
 285 carbonated mortars mixed with a w/c ratio of 1. For each samples, the specific attachment rate  
 286 is null during several days. This result confirms the existence of a latency period during which  
 287 no spot is fixed to the surface. Moreover, despite important uncertainties, influence of the  
 288 roughness on the algal attachment rate is obvious. Indeed, the rougher the mortar is, the  
 289 higher the attachment rate is.

290 The effect of the carbonation of mortars (roughness R2 and w/c = 1), on the algal  
 291 attachment rate is shown on Fig. 4. The attachment rate reached for the uncarbonated mortar  
 292 is much smaller than for the carbonated one.

293 For carbonated mortars and roughness R2, the influence of w/c ratio on the attachment  
294 rate is illustrated on Fig. 5. The results demonstrate that attachment specific rate for w/c ratios  
295 of 0.5 and 1 are very close. Thus, in our study, the effect of the porosity was negligible on the  
296 biofouling.

#### 297 298 4.1.2 Specific attachment rate constant 299

300 According to the Figs. 3, 4 and 5, beyond the latency time, the specific attachment rate,  
301 which is equivalent to the “nucleation” rate in the Avrami’s model, increases linearly with  
302 time. This result implies that the specific attachment rate can be modeled by a power law. In  
303 this case, the value of the power (q) is equal to 1 to express the attachment rate. Thus the  
304 specific attachment constant ( $k_g$ ) can be determined, beyond the latency time ( $t_l$ ), by a linear  
305 regression. Fig. 3 illustrates the fit obtained for carbonated mortars with a w/c ratio equal to 1.

306 The values of  $k_g$  for all the studied mortars are summarized in Table 3. For all the  
307 samples, to the exception of 05R3-C,  $k_g$  is strongly dependent of the roughness. Indeed, when  
308 the roughness increases,  $k_g$  also increases. For example,  $k_g$  of carbonated samples is 9 times  
309 higher and 30 times higher when the roughness increases from R1 to R2 and from R1 to R3  
310 respectively.

311 The behavior of the 05R3-C sample for which the specific attachment constant was  
312 smaller than the one obtained for 05R2-C was particular. Despite a high  $R_a$  value (186 $\mu$ m),  
313 the micro-roughness created by the sand grains was missing from the surface of these  
314 samples. This micro-roughness is important in promoting the algal adherence.

315 Moreover, beyond a roughness of 30 $\mu$ m, the relationship between  $k_g$  and the roughness  
316 ( $R_a$ ) seems to be linear (Fig. 6a). The slope of the straight line is around 4.6 and 0.7  
317 respectively for the carbonated samples and the uncarbonated ones. So the carbonated  
318 samples are more sensitive to the roughness than uncarbonated ones.

319 In addition, the carbonation, and then the surface pH, impacts the specific attachment  
320 constant. Indeed, whatever the roughness,  $k_g$  is always higher for carbonated samples than for  
321 uncarbonated ones.

#### 322 323 4.1.3 Latency time 324

325 The values of  $t_l$  are summarized in Table 3. The latency time decreased with the roughness  
326 and the carbonation. For carbonated samples, the effect of the roughness is weak (fig. 6b).  
327 However, the effect is strongly marked with uncarbonated mortars.

328 Moreover, the latency time is really dependent of the chemical state of the surface. Indeed,  
329 the carbonation of the mortars decreases the latency time and thus increases the  
330 biocompatibility of the substrate. For example, the latency times range from 6 to 10 days for  
331 carbonated samples and from 17 to 44 days for uncarbonated ones.

## 332 333 4.2. Experimental examination of growth process 334

335 Image analysis and specific algorithm enabled us to locate and track changes, during  
336 time, of the surface of each algal spot found on the surface of the sample. The evolution of the  
337 spot surface corresponds to the growth of algae and thus to a growth rate.

338 The growth rate constant ( $k_c$ ) was experimentally determined and calculated according to  
339 the equation (7).  
340

$$341 \quad k_c = \frac{\sqrt{S_{t+\Delta t}} - \sqrt{S_t}}{((t + \Delta t) - t)} \quad (7)$$



342  
343  
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345  
346

Where:

$S_t$  represents the area of an algal spot at time  $t$  ( $\mu\text{m}^2$ )

$S_{t+\Delta t}$  represents the area of the same algal spot at time  $t+\Delta t$  ( $\mu\text{m}^2$ )

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Fig. 7 shows the evolution of  $k_c$  with time for carbonated samples of w/c ratio equal to 0.5. The values of  $k_c$  were determined for colonization rates below 50 %. Beyond this value, the number of algal spots that could be isolated to monitor their growth became too low. By considering the high dispersion of experimental results, the growth rate constant ( $k_c$ ) does not seem to vary significantly with time. This remark is also valid for all other mortars.

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If the growth rate constant is considered as independent of time, the average of this constant could be calculated for each material. The results are presented on Fig. 8. Experimental errors were very important. It is therefore difficult to highlight the effect of intrinsic characteristics of materials on the growth rate constant. However, despite these uncertainties, it seems that, for each type of sample, the algal growth was lower on the uncarbonated samples than on the carbonated ones.

358

### 359 4.3. Simulation of colonization rates

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In order to simulate the colonization process and in accordance with the results of paragraph 4.1.2,  $q$  was fixed to 1 and thus  $n$  was equal to 4. The  $K$  parameter was adjusted from the equation (3) by the least squares method ( $K$  was determined so that the error was minimal). In order to express the deviation between the model and experimental data, a fiability factor ( $R$ ) was calculated as follow:

367

$$R = \sqrt{\frac{\sum_{t=1}^m (X_{st} - X_{et})^2}{\sum_{t=1}^m X_{et}^2}} \quad (8)$$

368  
369  
370  
371  
372

Where:

$X_{st}$  represents the simulated colonization rate at time  $t$

$X_{et}$  represents the experimental colonization rate at time  $t$

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All the values of  $K$  are summarized in the Table 3. As expected,  $K$  increases with the roughness and the carbonation with the exception of sample 05C-R3 (w/c=0.5, carbonated and high roughness). This result was previously described and discussed in paragraph 4.1.2. By using the equation (3) and the values of  $K$ , it was possible to simulate the colonization rate. For all the carbonated mortars, the simulated curves were close to the experimental points (Fig. 9). The fiability factors were small with a maximum error lower than 11 %. For most of the uncarbonated mortars, a good agreement between simulated colonization rate and experimental data was obtained (Fig. 10). However, divergences can be noticed for the 10UC-R2 serie (w/c=1, uncarbonated and intermediate roughness) which was not well simulated. Indeed, the fiability factor is equal to 25%. However, as shown on fig. 10, these mortars exhibited abnormal behavior in our experiments.

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### 385 4.4. Comparison between the experimental and the calculated growth rate constant

388 From the experimental values of  $k_g$  determined by image analysis, the values of the  
389 growth rate constant ( $k_{c\text{-calculated}}$ ) were calculated by the equation (9). Results obtained were  
390 compared with those experimentally determined.  
391

$$392 \quad k_{c\text{-calculated}} = \sqrt{\frac{K}{Ak_g}} \quad (9)$$

393  
394 As shown in Fig. 11, for all mortars, calculated values were higher than experimental  
395 ones. Except in the case of mortar 05UC-R1, for which the calculated value reached up 4  
396 times the experimental one, the ratios between  $k_{c\text{-calculated}}$  and  $k_{c\text{-experimental}}$  were inferior to 3 for  
397 all the cases. Taking into account the uncertainties, the two values remained nevertheless  
398 close.  
399

## 400 5. Discussion

401  
402 In this paper, it was demonstrated that the Avrami's theory could be applied in order to  
403 simulate the colonization kinetic of mortar surfaces by algae, in an accelerated laboratory test.  
404 For almost all mortars, the simulated curves are very close to the experimental results. The  
405 nucleation-growth mechanism is well adapted to describe the phenomenon of biological  
406 colonization. Indeed, the colonization started by attachment of small algal spots (called  
407 "nuclei" in Avrami's law) and is extended by the growth of these latter. Each of these two  
408 processes was directly analyzed by image analysis.

409 The algal attachment on a surface is particularly complex, depending on several  
410 parameters such as the microorganism nature, the substrate type, the medium type, the  
411 microorganism concentration, which modify the interactions between the substrate and  
412 microorganisms and microorganisms themselves. However, in the case of this work, the  
413 mechanism of attachment was not studied. The author focused on the macroscopic aspect of  
414 the algal adhesion. Indeed, the size of the smallest area of algal spot which could be detected  
415 by image analysis was approximately  $7200 \mu\text{m}^2$ . This area corresponded to around 80 times  
416 the surface of an algal cell. So, a spot is composed of a lot of algal cells. This spot concept  
417 integrates the complex phenomena involved in the adhesion of cells to a surface, the cell  
418 density and the surface properties which depend on metabolic activity and physiological state,  
419 necessary to retention and self-organization of cells (Carnazza et al. 2011).  
420

421 The algal attachment on the sample surface was significantly influenced by the  
422 roughness. Indeed, roughness provides asperities, which promoted the anchorage of algal  
423 cells. It results in a higher specific attachment rate and a shorter latency time.

424 The carbonation, by decreasing surface pH, favored the attachment and growth processes.  
425 It produced the same effects than the roughness, i.e. increasing the specific attachment rate  
426 and shortening the latency time. However, unlike the roughness which acts physically on the  
427 ability of algae to cling on a substrate, the carbonation may affect the algal metabolism.  
428 Indeed, in the case of carbonated mortars, the algal cells are in a less alkaline medium, and so,  
429 less stressful conditions than in the case of uncarbonated ones. The ability of attaching and  
430 spreading of algae on the surface are thus better for carbonated samples (Tran et al. 2012).  
431 The growth of the spots is the consequence of the vegetative or cellular multiplication which  
432 is favored at low pH (Škaloud, 2006).  
433

434 The attachment of algae on the surface of the substrate is governed by adaptive metabolic  
435 interactions between algal cells and the substrate (Fattom et Shilo 1984, Finlay et al. 2002,

436 Barberousse 2006). The algal extracellular polysaccharides can play the role of glue (Robins  
437 et al. 1986, Gantar et al. 1995, Barberousse 2006). These polymers are involved in the initial  
438 contact between the cell and the surface, and act over time (Barberousse 2006). Indeed, they  
439 exist permanently on the cell wall and are adsorbed on the surface in contact.

440 These metabolites are composed of hexoses (glucose, galactose, mannose), 6-  
441 deoxyhexoses (rhamnose, fucose) and pentoses (xylose, arabinose). The main constituent  
442 depends on the algal species considered and the substrate. For *Klebsormidium flaccidum*,  
443 mannose was identified as the major component (Barbarosse 2006).

444

445 In contrast, the role of w/c ratio on the algal attachment was not detected. As mentioned  
446 by Tran et al. (2012), due to the test conditions, the mortars permanently contained abundant  
447 water. So this last was not a limited factor for the algal growth.

448

449 It was proved by image analysis that, in the early stage, algal specific attachment rate  
450 evolved linearly with time. In order to model the colonization rate (eq. (3)), it was supposed  
451 that the attachment rate increased linearly with time, throughout all the process.

452

453 The influence of intrinsic material characteristics on the growth process has not been  
454 shown due to important experimental uncertainties. The errors may result from the  
455 detachment of ancient cells or the adherence of new cells at the periphery of existing spots  
456 due to the periodic runoff. In these cases, the surface variation of spots was no longer only  
457 related to their growth. However, the assumption of constant growth rate over time was found  
458 acceptable.

459 The variation in algal activity between the different tests prevents us from generalizing  
460 the results. Thus, no general equation describing the relationship between the kinetic  
461 parameters ( $t_i$ ,  $k_g$ ) and the intrinsic material parameters could be obtained.

462

## 463 **6. Conclusion**

464

465 The kinetic of biological colonization on mortar surface was well modeled in applying  
466 Avrami's theory. The experimental conditions used in this study were suitable for the  
467 application of the Avrami's model and satisfied to assumptions of the model. The model  
468 based on two steps, "nucleation" or attachment and growth, closely represents the  
469 colonization rate.

470 The influence of roughness and carbonation on the attachment frequency was  
471 highlighted. These two intrinsic parameters promoted the anchorage of algae on the substrate  
472 by increasing the attachment rate and by shortening the latency time. The porosity had no  
473 effect in our tests.

474 According to the results, the evolution of the attachment rate as function of time seems  
475 linear. So the attachment rate followed a powerful law with a power  $q$  equal to 1.

476 Due to large experimental uncertainties, it was difficult to conclude on the growth rate of  
477 algal spots. However, supposition of a constant growth rate of spots appeared acceptable.

478

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Table 1. Mortar formulation

Component	Cement	Sand	Calcareous Filler	Admixture <sup>a</sup> (in the case of w/c = 1)
% mass of dry mixture	30	65	5	0.27

572

<sup>a</sup> in addition to dry mixture (cement, sand and filler)

573

574

575

576

Table 2. Characteristics of the mortars

577

	Ratio w/c	Porosity (%)	Surface pH	Code	R <sub>a</sub> (μm)	
Carbonated	0.5	10.6 ± 0.4	9.5 ± 0.2	Roughness 1	05C-R1	40 ± 9
				Roughness 2	05C-R2	90 ± 8
				Roughness 3	05C-R3	186 ± 21
	1.0	32.1 ± 1.9	9.0 ± 0.1	Roughness 1	10C-R1	30 ± 3
				Roughness 2	10C-R2	55 ± 4
				Roughness 3	10C-R3	169 ± 17
Uncarbonated	0.5	15.9 ± 0.6	11.2 ± 0.4	Roughness 1	05UC-R1	29 ± 5
				Roughness 2	05UC-R2	47 ± 6
				Roughness 3	05UC-R3	123 ± 9
	1.0	37.2 ± 0	11 ± 0.4	Roughness 1	10UC-R1	29 ± 5
				Roughness 2	10UC-R2	55 ± 4
				Roughness 3	10UC-R3	123 ± 9

578

579

580

581

Table 3. Experimental and calculated kinetic parameters and fiability factor

582

	Mortar	t <sub>l</sub> (day)	k <sub>g</sub> (×10 <sup>10</sup> ) (spot/μm <sup>2</sup> .day <sup>2</sup> )	k <sub>c</sub> -experimental (μm/day)	K (×10 <sup>6</sup> ) (spot/day <sup>4</sup> )	R (%)	k <sub>c</sub> -calculated (μm/day)
Carbonated	10C-R1	10 ± 2	21.3	184 ± 147	22.3	5	299
	10C-R2	8 ± 1	183.9	122 ± 93	193.6	7	350
	10C-R3	6 ± 1	652.6	109 ± 71	437.9	7	285
	05C-R1	9 ± 2	39.0	132 ± 103	36.2	11	337
	05C-R2	8 ± 1	376.2	101 ± 87	492.5	10	298
	05C-R3	8 ± 2	127.0	124 ± 95	76.4	8	263
Uncarbonated	10UC-R1	27 ± 1	1.5	74 ± 68	0.2	8	131
	10UC-R2	20 ± 1	84.1	66 ± 51	1.5	25	103
	10UC-R3	18 ± 2	86.3	83 ± 57	31.8	12	201
	05UC-R1	44 ± 5	1.9	60 ± 50	0.7	8	237
	05UC-R2	27 ± 4	8.4	74 ± 47	0.4	8	72

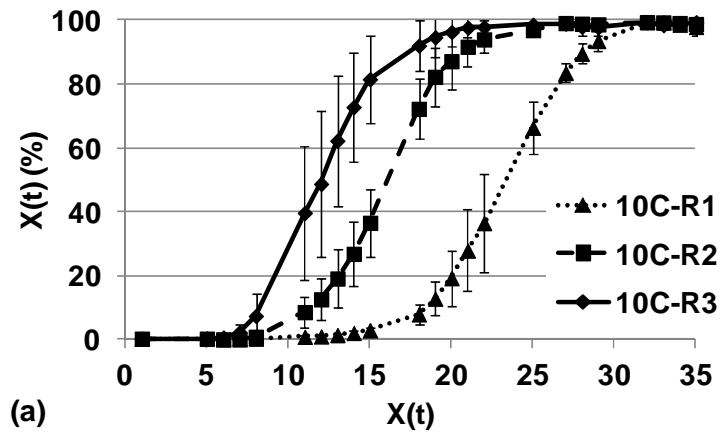
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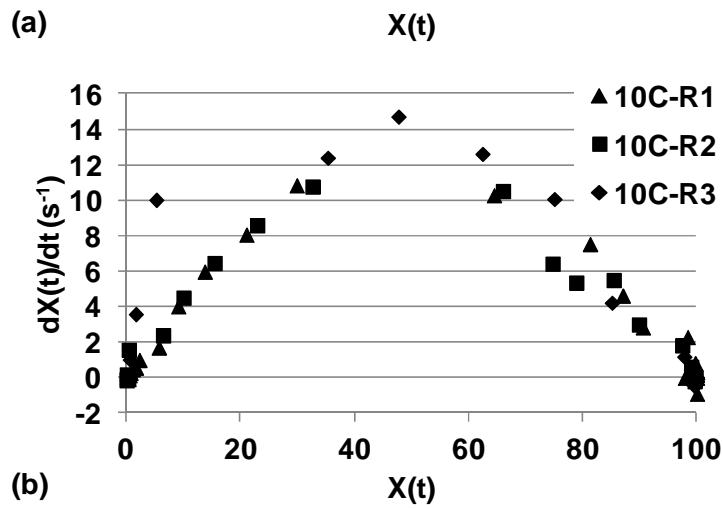
05UC-R3	$17 \pm 3$	66.1	$83 \pm 58$	32.7	12	178
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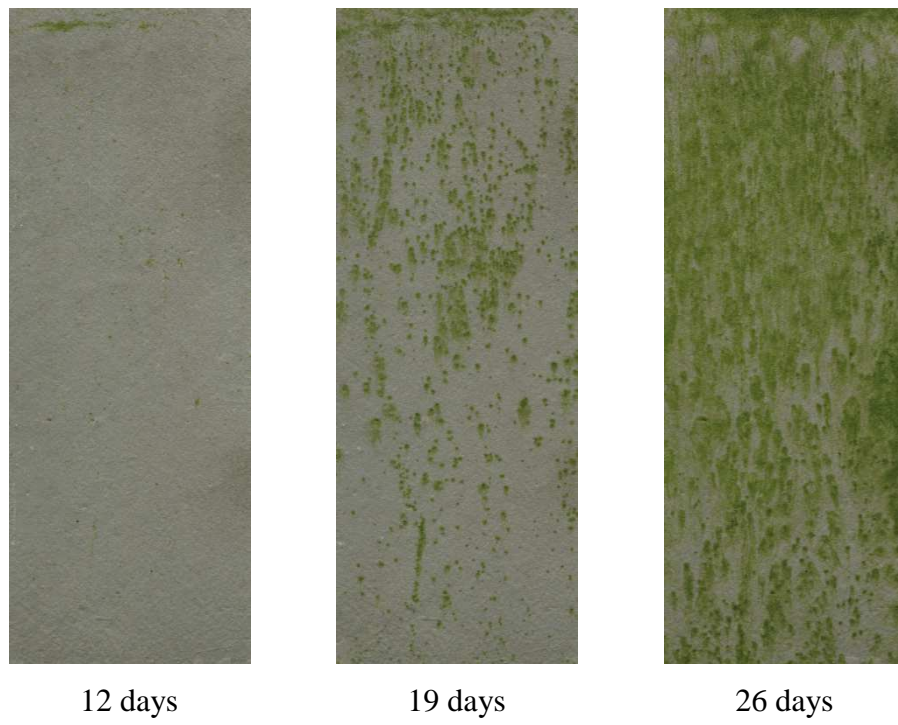
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587 Fig. 1 Influence of roughness ( $R_1=30\mu m$ ,  $R_2=55\mu m$  and  $R_3=169\mu m$ ) on the algal biofouling

588 of carbonated (C) mortars with a w/c ratio equal to 1: a)  $X(t)$  and b)  $dX(t)/dt$

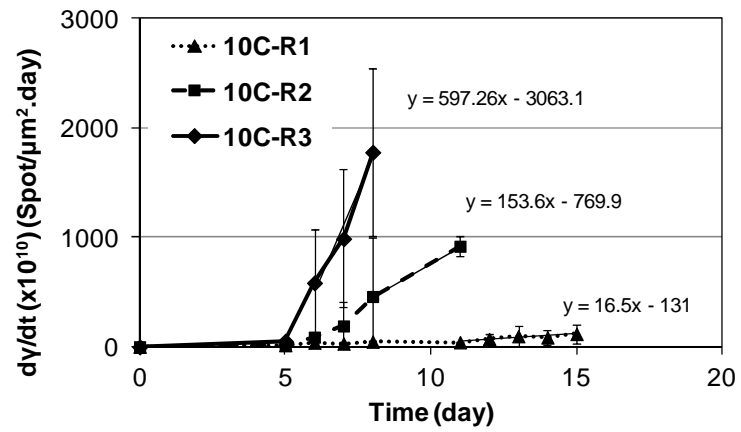
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591 Fig. 2 Surface of carbonated mortars with a w/c ratio of 0.5 and a roughness R1, colonized by  
592 algae over time  
593

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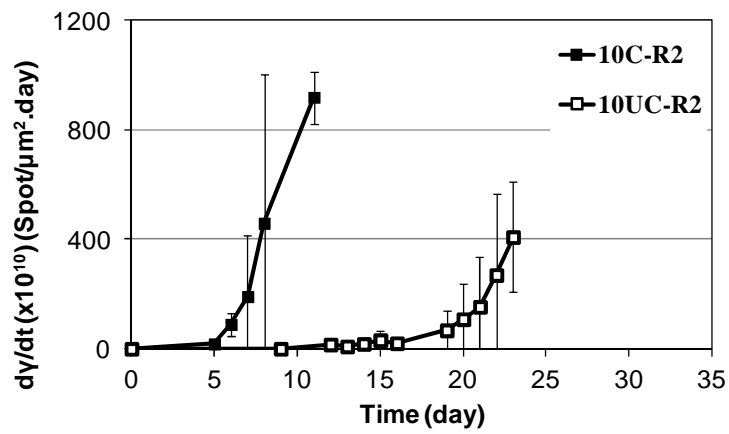
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Fig. 3 Influence of the roughness (R1, R2 and R3) on the specific attachment rate of algae

597

(specifications of mortars: carbonated (C) and w/c ratio of 1)

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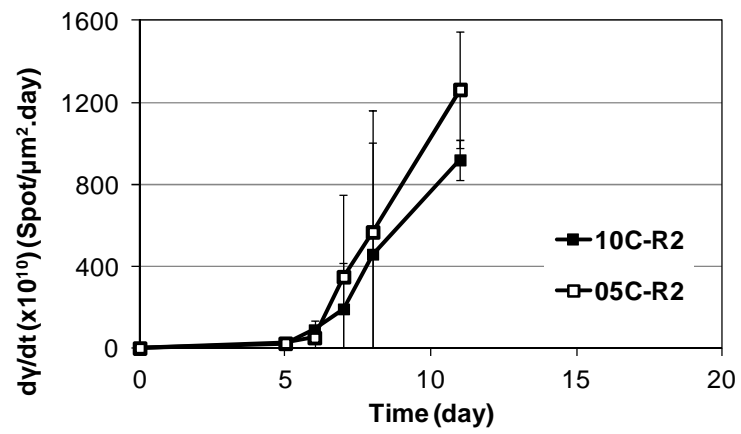
601 Fig. 4 Influence of the carbonation state (C for carbonated and UC for uncarbonated) on

602 the specific attachment rate of algae (specifications of mortars: intermediate roughness (R2),

603 w/c ratio of 1)

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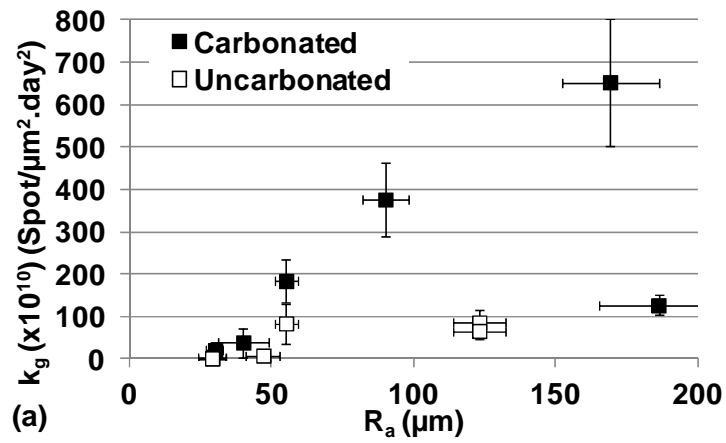
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607 Fig. 5 Influence of the w/c ratio on the specific attachment rate of algae (specifications of

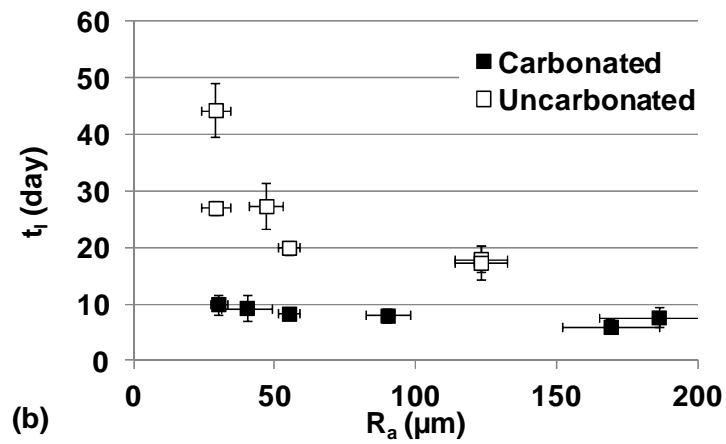
608 mortars: carbonated (C) and intermediate roughness (R2))

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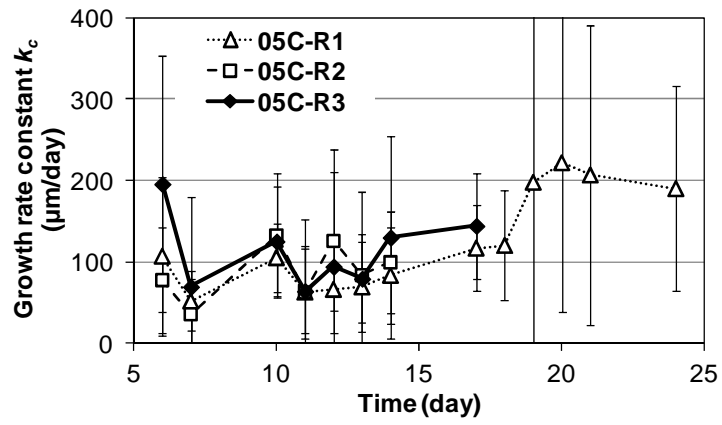
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Fig. 6 Effect of roughness ( $R_a$ ) on the specific attachment constant (a) and on the latency time (b) for carbonated (C) and uncarbonated (UC) mortars

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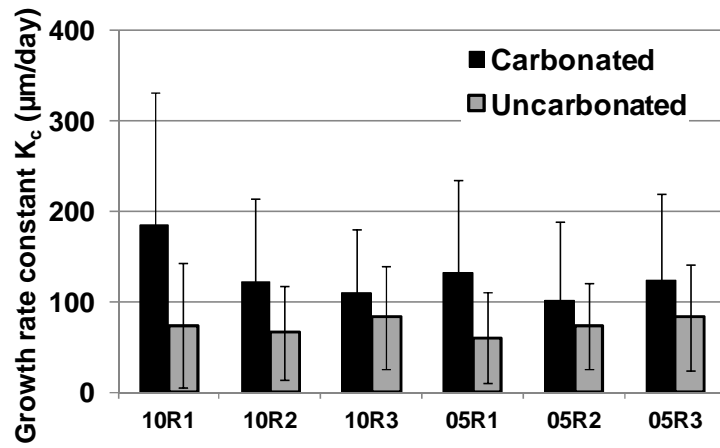
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Fig. 7 Influence of roughness (R1, R2 and R3) on the growth rate constant versus time  
(specifications of mortars: carbonated (C) and w/c ratio of 0.5)

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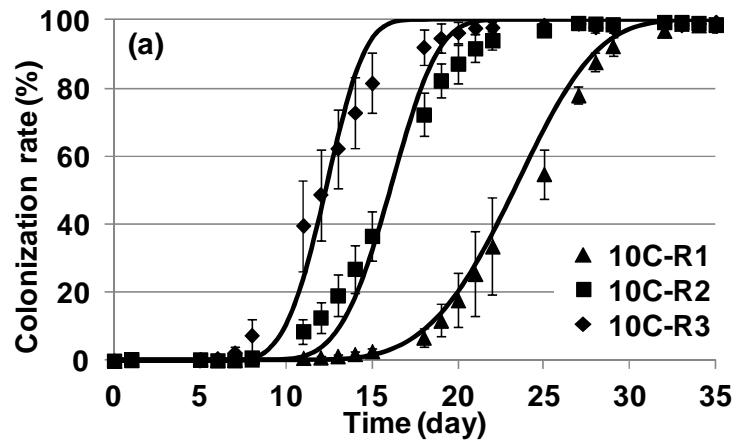
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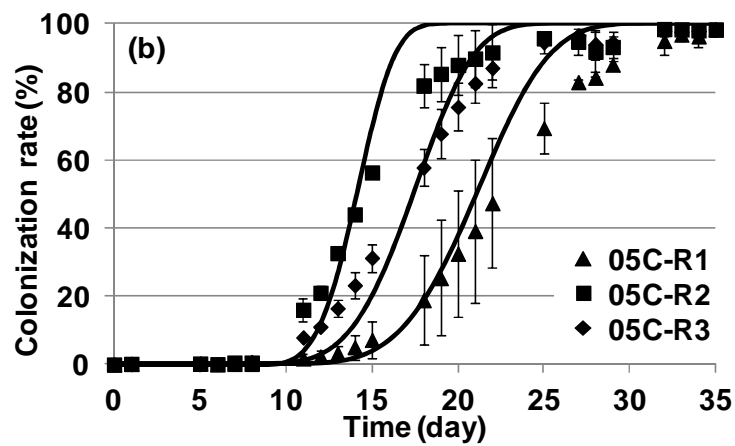
Fig. 8 Average of the growth rate constant for all the studied samples

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628 Fig. 9 Simulation of colonization rate for carbonated mortars mixed with w/c ratio of 1 (a) and

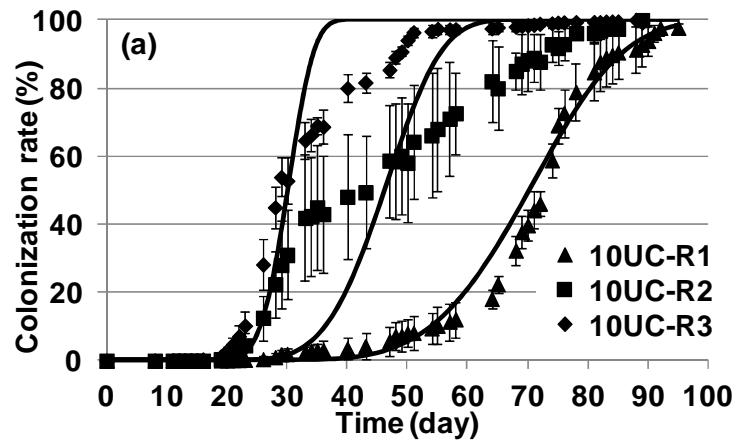
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0.5 (b) (points: experimental data; lines: simulation)

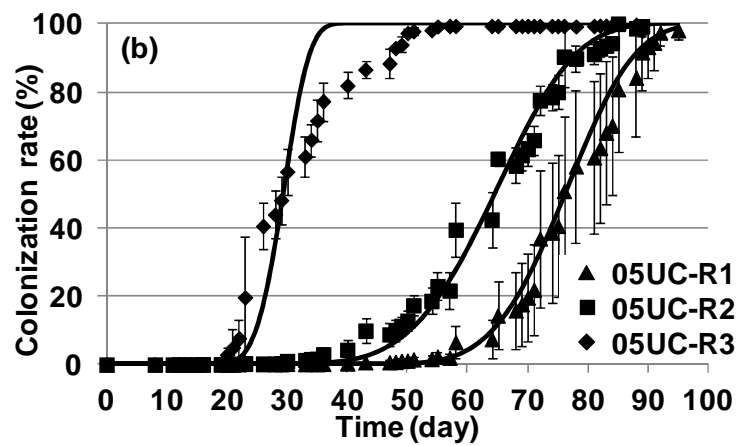
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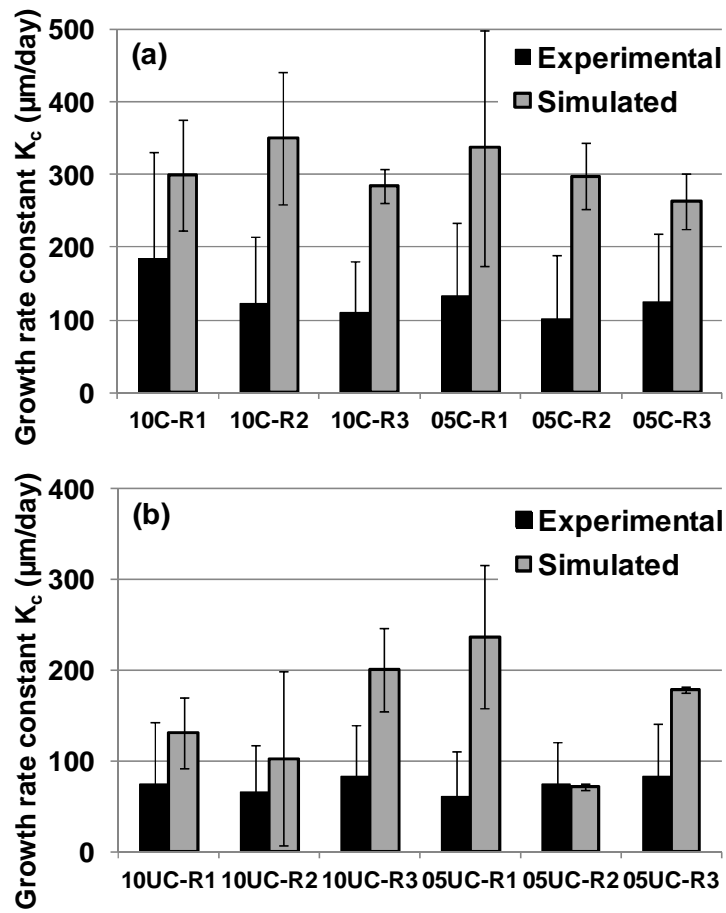
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634 Fig. 10 Simulation of colonization rate for uncarbonated mortars mixed with w/c ratio of

635 1 (a) and 0.5 (b) (points: experimental data; lines: simulation)

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Fig. 11 Comparison between the experimental and the calculated growth rate constants for carbonated (a) and uncarbonated (b) mortars