



HAL
open science

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

Thu Hien Tran, Alexandre Govin, René Guyonnet, Philippe Grosseau, Christine Lors, Denis Damidot, Olivier Devès, Bertrand Ruot

► To cite this version:

Thu Hien Tran, Alexandre Govin, René Guyonnet, Philippe Grosseau, Christine Lors, et al.. Avrami's law based kinetic modeling of colonization of mortar surface by alga *Klebsormidium flaccidum*. *International Biodeterioration and Biodegradation*, 2013, 79, pp.73-80. 10.1016/j.ibiod.2012.12.012 . hal-00804718

HAL Id: hal-00804718

<https://hal.science/hal-00804718v1>

Submitted on 26 Mar 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3
4
5 Thu Hien TRAN^{a,b,c}, Alexandre GOVIN^a, René GUYONNET^a, Philippe GROSSEAU^a,
6 Christine LORS^{b,c}, Denis DAMIDOT^{b,c}, Olivier DEVES^d, Bertrand RUOT^d

7
8 ^a: Ecole Nationale Supérieure des Mines de Saint Etienne, SPIN-EMSE,
9 CNRS:UMR5307, PROPICE, LGF, 158 cours Fauriel, F-42023 St-Etienne, France

10 ^b: Université Lille Nord de France, 1 bis Georges Lefèvre, F-59044 Lille, France

11 ^c: Ecole Nationale Supérieure des Mines de Douai, LGCgE-GCE, 941 Rue Charles
12 Bourseul, F-59508 Douai, France

13 ^d: Université Paris Est, Centre Scientifique et Technique du Bâtiment, Département
14 Enveloppe et Revêtements, 84 avenue Jean Jaurès, Champs-sur-Marne,
15 F-77447 Marne-la-Vallée, France

16
17
18
19 **Nomenclature**

20 X(t) colonization rate (%)
21 t time (day)
22 t_l latency time (day)
23 γ number of algal spots at time t per unit area
24 dy/dt specific attachment rate (Spot/ μm^2 .day)
25 S surface of an algal spot (μm^2)
26 k_g specific attachment constant (Spot/ μm^2 .day²)
27 k_c growth rate constant ($\mu\text{m}/\text{day}$)
28 K overall rate constant (Spot/day⁴)
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 ± 1 °C and 95 ± 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 CO₂, at 21 ± 1 °C, and 65 ± 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 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[®]
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[®] software. The objective of the algorithm
165 was to quantify new spots appearing between time t and t+ Δt by comparison of the two
166 images acquired respectively at time t and t+ Δt . 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 γ 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 θ 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 μ 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 μ 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).

$$340 \quad k_c = \frac{\sqrt{S_{t+\Delta t}} - \sqrt{S_t}}{((t + \Delta t) - t)} \quad (7)$$

342
343
344
345
346

Where:

S_t represents the area of an algal spot at time t (μm^2)

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

347
348
349
350
351

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.

352
353
354
355
356
357

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

360
361
362
363
364
365
366

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

373
374
375
376
377
378
379
380
381
382
383

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.

384

385 4.4. Comparison between the experimental and the calculated growth rate 386 constant

387

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

479 **References**

480 Ariño, X., Gomez-Bolea, A., Saiz-Jimenez, C., 1997. Lichens on ancient mortar.
481 *International Biodeterioration and Biodegradation* 40, 217-224.

482 Avrami, M., 1939. Kinetics of phase change I - General Theory. *Journal of Chemical*
483 *Physics* 7, 1103-1112.

484 Avrami, M., 1940. Kinetics of phase change II - Transformation-Time Relations for
485 *Random Distribution Nuclei*. *Journal of Chemical Physics* 8, 212-224.

486 Avrami, M., 1941. Granulation, phase change, and microstructure - Kinetics of phase
487 change III. *Journal of Chemical Physics* 9, 177-185.

488 Barberousse, H., Tell, G., Yéprémian, C., Couté, A., 2006. Diversity of algae and
489 cyanobacteria growing on building façades in France. *Algological Studies* 120: 83-110.

490 Carnazza, S., Marletta, G., Frasca, M., Fortuna, L., Guglielmino, S., 2011. Spatial
491 patterns of microbial retention on polymer surfaces. *Journal of Adhesion Science and*
492 *Technology* 25, 2255-2280

493 Crispim, C.A., Gaylarde, P.M., Gaylarde, C.C., 2003. Algal and cyanobacterial biofilms
494 on calcareous historic buildings. *Current Microbiology* 46, 79-82.

495 Delmon, B., 1969. *Introduction à la cinétique hétérogène*. Ed. Technip, Paris.

496 Deruelle, S., 1991. Rôle du support dans la croissance des microorganismes. *Materials*
497 *and Structures* 24, 163-168.

498 Fattom, A., Shilo, M., 1984. Hydrophobicity as an adhesion mechanism of benthic
499 cyanobacteria. *Applied and Environmental Microbiology* 47, 135-143.

500 Finlay, J.A., Callow, M.E., Ista, L.K., Lopez, G.P., Callow, J.A., 2002. The Influence of
501 Surface Wettability on the Adhesion Strength of Settled Spores of the Green Alga
502 *Enteromorpha* and the Diatom *Amphora*. *Integrative and Comparative Biology* 42, 1116-
503 1122.

504 Galwey, A.K., Brown, M.E., 1999 *Thermal Decomposition of Ionic Solids*, Elsevier,
505 Netherlands.

506 Gantar, M., Rowell, P., Kerby, N.W., 1995. Role of extra- cellular polysaccharides in the
507 colonization of wheat (*Triticum vulgare* L.) roots by N₂-fixing cyanobacteria. *Biology and*
508 *Fertility of Soils* 19, 41-48.

509 Gaylarde, P.M., Gaylarde, C.C., 2000. Algae and cyanobacteria on painted buildings in
510 Latin America. *International Biodeterioration and Biodegradation* 46, 93-97.

511 Gaylarde, C.C., Gaylarde, P.M., 2005. A comparative study of the major microbial
512 biomass of biofilms on exteriors of buildings in Europe and Latin America. *International*
513 *Biodeterioration and Biodegradation* 55, 131-139.

514 Grant, C., 1982. Fouling of terrestrial substrates by algae and implications for control – a
515 review. *International Biodeterioration Bulletin* 18, 57-65.

516 Hay, J. N., 1971. Application of the modified avrami equations to polymer crystallisation
517 kinetics. *British Polymer Journal* 3, 74–82.

518 John, D.M., 1988. Algal growth on buildings: A general review and methods of
519 treatment. *Biodeterioration Abstracts* 2, 81-102.

520 Joshi, C.D., Mukunda, U., 1997. Algal disfigurement and degradation of architectural
521 paints in India. *Paintindia* 47, 27-32.

522 Johnson, W.A., Mehl, R.F., 1939. Reaction Kinetics in Processes of Nucleation and
523 Growth. *Trans. Amer. Inst. Ming. Metall.* 135, 416-458.

524 Miller, A., Dionísio, A., Macedo, M.F., 2006. Primary bioreceptivity: A comparative
525 study of different Portuguese lithotypes. *International Biodeterioration and Biodegradation*
526 57, 136-142.

527 Miller, A.Z., Dionísio, A., Laiz, L., Macedo, M.F., Saiz-Jimenez, C., 2009. The influence
528 of inherent properties of building limestones on their bioreceptivity to phototrophic
529 microorganisms. *Annals of Microbiology* 59, 705-713.

530 Ohshima, A., Matsui, I., Yuasa, N., Henmi, Y., 1999. A study on growth of fungus and
531 algae on mortar. *Transactions of the Japan Concrete Institute* 21, 173-178.

532 Ortega-Calvo, J.J., Hernandez-Marine, M., Saiz-Jimenez, C., 1991. Biodeterioration of
533 building materials by cyanobacteria and algae. *International Biodeterioration* 28, 165-185.

534 Ortega-Calvo, J.J., Sanchez-Castillo, P.M., Hernandez-Marine, M., Saiz-Jimenez, C.,
535 1993. Isolation and characterization of epilithic chlorophytes and cyanobacteria from two
536 Spanish cathedrals (Salamanca and Toledo). *Nova Hedwigia* 57, 239-253.

537 Ortega-Calvo, J.J., Ariño, X., Hernandez-Marine, M., Saiz-Jimenez, C., 1995. Factors
538 affecting the weathering and colonization of monuments by phototrophic microorganisms.
539 *Science of The Total Environment* 167, 329-341.

540 Pietrini, A.M., Ricci, M., Bartolini, M., Giuliani, M.R., 1985. A reddish colour alteration
541 caused by algae on stoneworks. *Proceedings of the Vth international congress on deterioration
542 and conservation of stone*, Presses polytechniques romandes, Lausanne, 653-662.

543 Prieto, B., Silva, B., 2005. Estimation of the potential bioreceptivity of granitic rocks
544 from their intrinsic properties. *International Biodeterioration and Biodegradation* 56, 206-215.

545 Rindi, F., Guiry, M.D., López-Bautista, J.M., 2008. Distribution, morphology, and
546 phylogeny of *Klebsormidium* (*Klebsormidiales*, *Charophyceae*) in urban environments in
547 Europe. *Journal of Phycology* 44, 1529-1540.

548 Robins, R.J., Hall, D.O., Shi, D.J., Turner, R.J., Rhodes, M.J.C., 1986. Mucilage acts to
549 adhere cyanobacteria and cultured plant cells to biological and inert surfaces. *FEMS
550 Microbiology Letters* 34, 155-160.

551 Ruot, B., Barberousse, H. (2007). Quantification and kinetic modeling of the colonisation
552 of façade rendering mortars by algae. VII SBTa, Recife, Brazil, 1-12.

553 Škaloud, P., 2006. Variation and taxonomic significance of some morphological features
554 in european strains of *Klebsormidium* (*Klebsormidiophyceae*, *Streptophyta*). *Nova Hedwigia*
555 83, 533-550.

556 Slováček, M., 2004. Application of numerical simulation of heat treatment in industry. *J.
557 Phys. IV France* 120, 753-760.

558 Tomaselli, L., Lamenti, G., Bosco, M., Tiano, P., 2000. Biodiversity of photosynthetic
559 micro-organisms dwelling on stone monuments. *International Biodeterioration and
560 Biodegradation* 46, 251-258.

561 Tran, T.H., Govin, A., Guyonnet, R., Grosseau, P., Lors, C., Garcia-Diaz, E., Damidot,
562 D., Deves, O., Ruot, B., 2012. Influence of the intrinsic characteristics of mortars on
563 biofouling by *Klebsormidium flaccidum*. *International Biodeterioration and Biodegradation*
564 70, 31-39.

565 Wee, Y.C., Lee K.B., 1980. Proliferation of algae on surfaces of buildings in Singapore.
566 *International Biodeterioration Bulletin* 16, 113-117.

567
568

569

570

571

Table 1. Mortar formulation

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

572

^a 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 _a (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

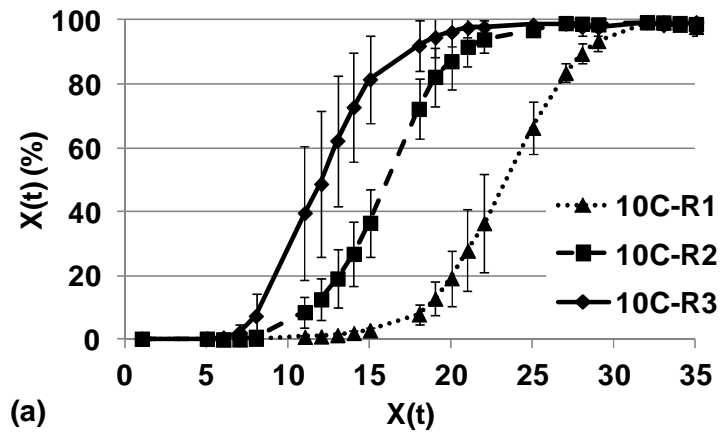
582

	Mortar	t _l (day)	k _g (×10 ¹⁰) (spot/μm ² .day ²)	k _c -experimental (μm/day)	K (×10 ⁶) (spot/day ⁴)	R (%)	k _c -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

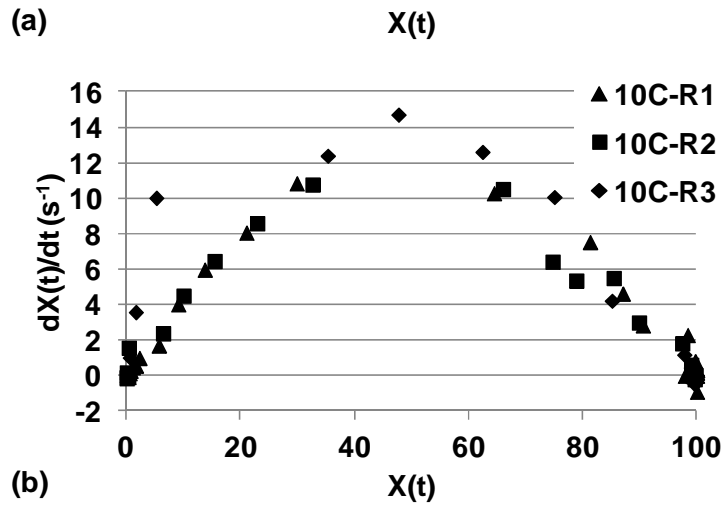
583

05UC-R3	17 ± 3	66.1	83 ± 58	32.7	12	178
---------	------------	------	-------------	------	----	-----

584



585

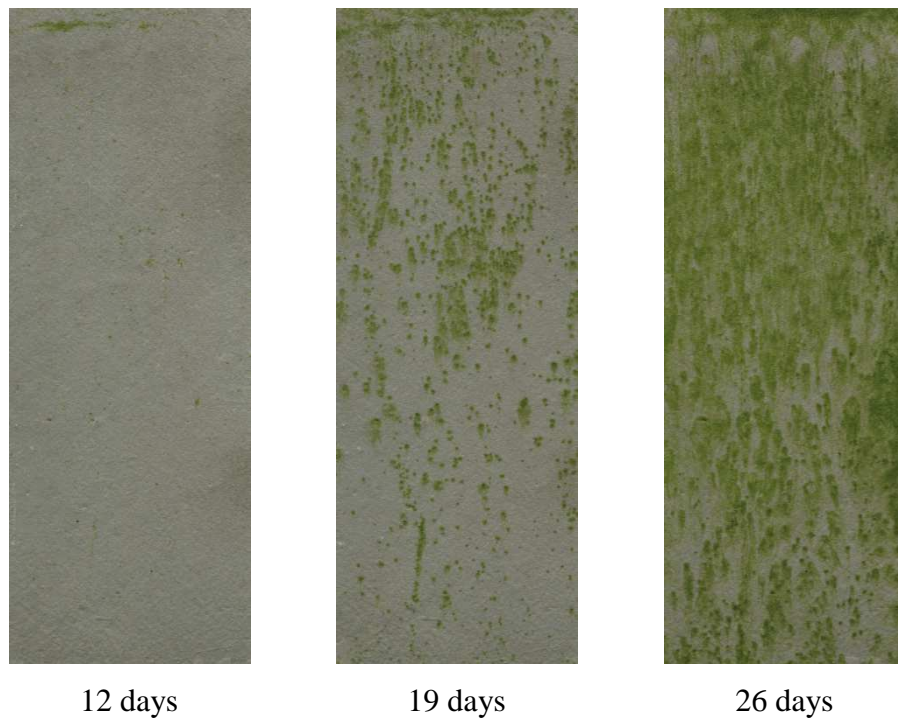


586

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

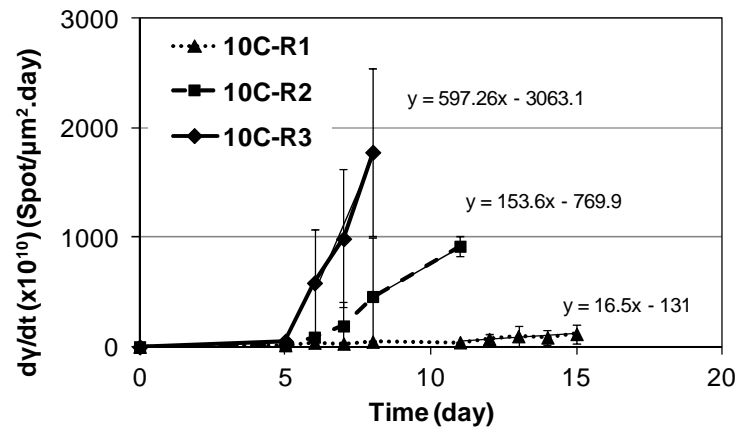
588 of carbonated (C) mortars with a w/c ratio equal to 1: a) $X(t)$ and b) $dX(t)/dt$

589



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

594



595

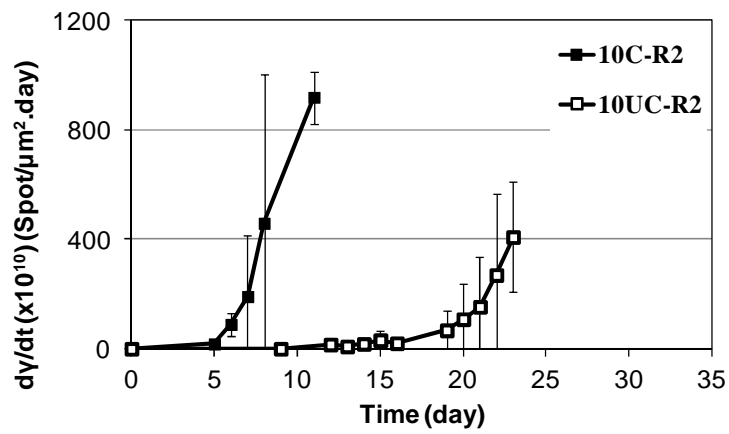
596

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)

598



600

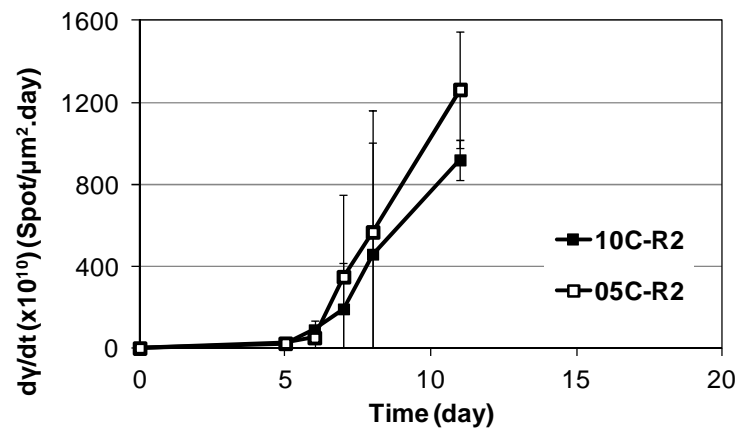
601 Fig. 4 Influence of the carbonation state (C for carbonated and UC for uncarbonated) on

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

603 w/c ratio of 1)

604

605



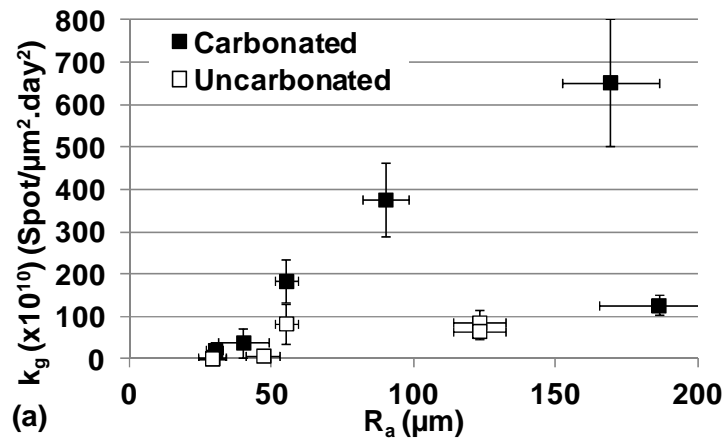
606

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

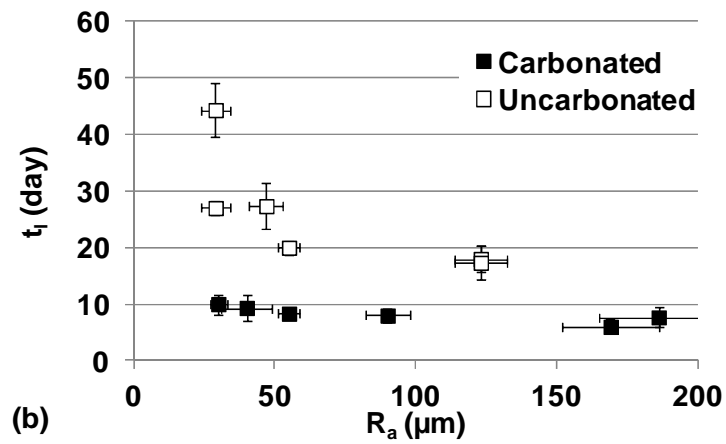
608 mortars: carbonated (C) and intermediate roughness (R2))

609

610



611



612

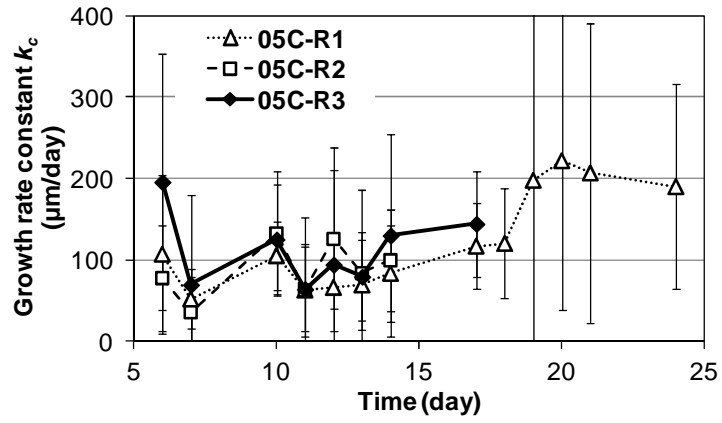
613

614

615

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

616



617

618

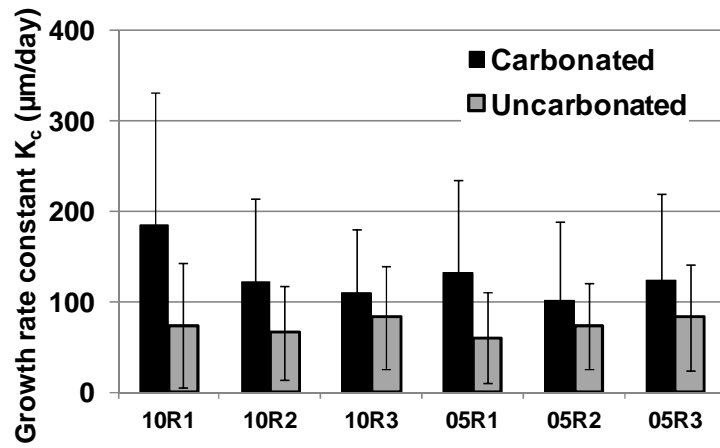
Fig. 7 Influence of roughness (R1, R2 and R3) on the growth rate constant versus time

619

(specifications of mortars: carbonated (C) and w/c ratio of 0.5)

620

621



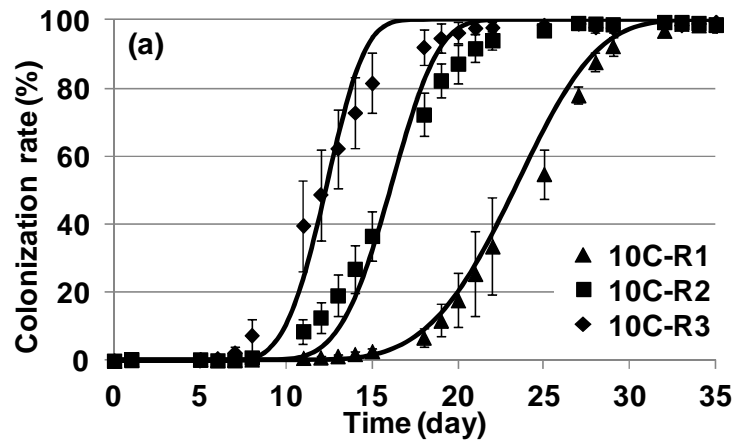
622

623

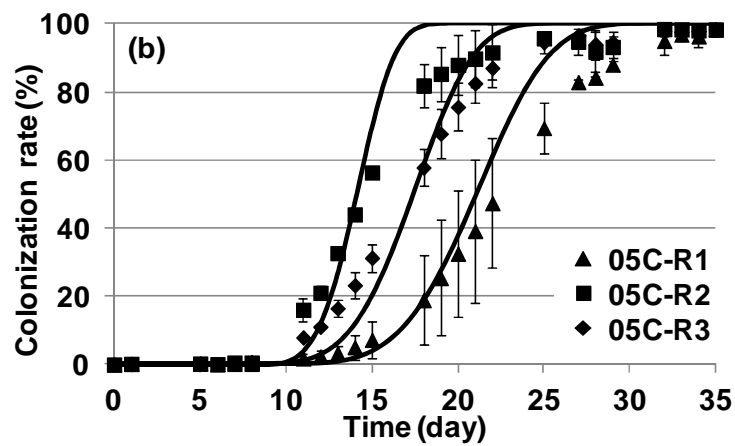
Fig. 8 Average of the growth rate constant for all the studied samples

624

625



626



627

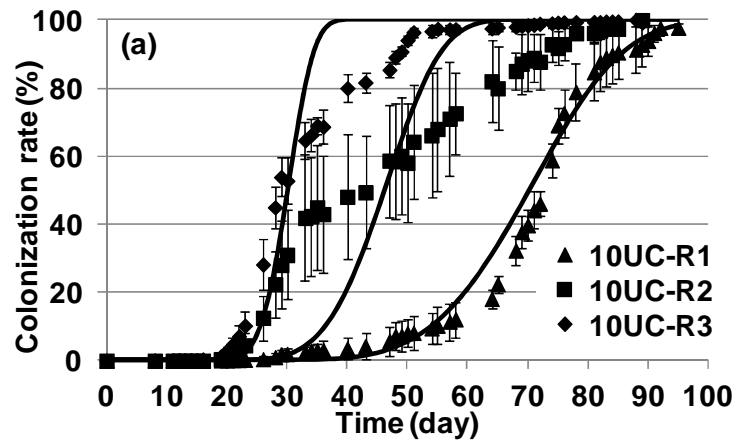
628 Fig. 9 Simulation of colonization rate for carbonated mortars mixed with w/c ratio of 1 (a) and

629

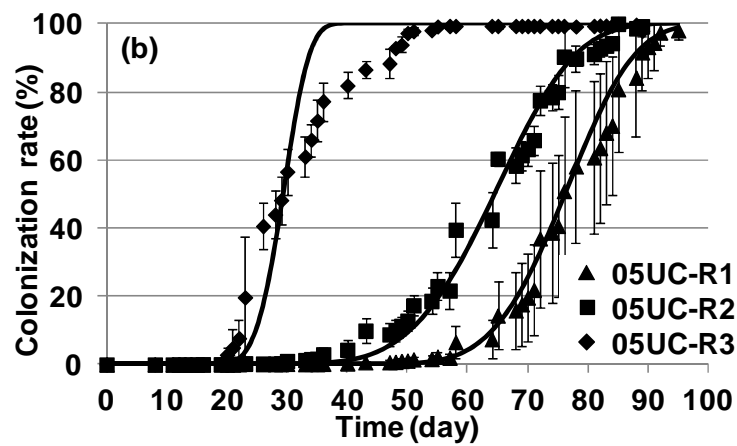
0.5 (b) (points: experimental data; lines: simulation)

630

631



632



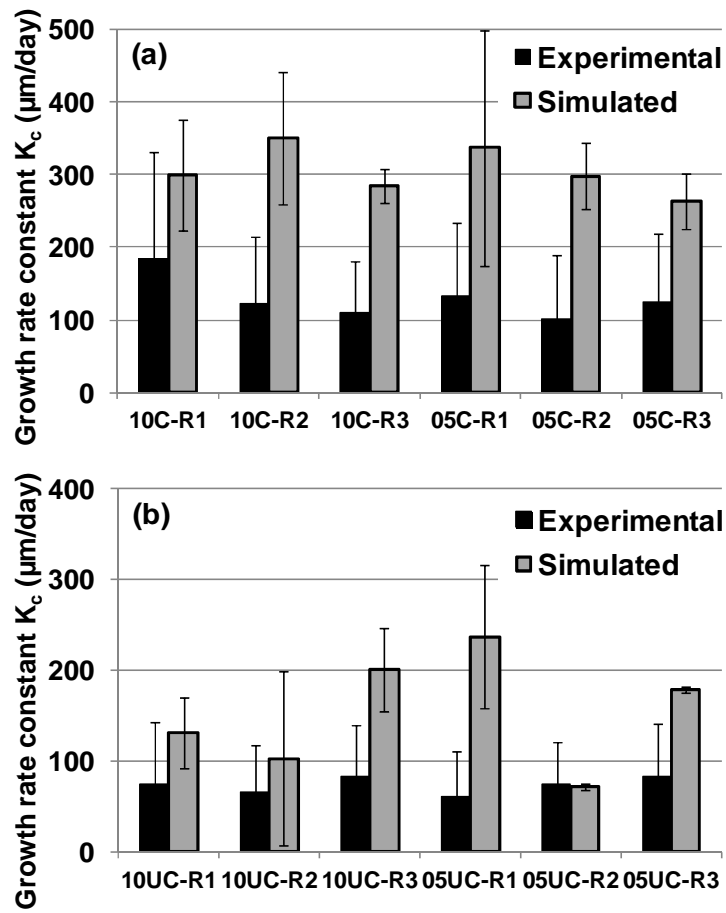
633

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)

636

637



638

639

640

641

642

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