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Ability in biofouling by <I>*Klebsormidium flaccidum*</I> of mortars: Influence of the intrinsic characteristics

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ABSTRACT:
The aim of this study was to highlight the influence of the intrinsic properties of mortars (roughness, porosity, and surface pH) on their susceptibility to biodegradation by phototrophic microorganisms. An accelerated fouling test was performed using the green algae <I>*Klebsormidium flaccidum*</I>. The biofouling was evaluated by means of image analysis. The colonization of samples was not influenced by the porosity because of the specific conditions of testing. However, the increase in roughness and the decreases in surface pH by the means of carbonation, promote algal development significantly. Thus the latency time is shortened and the rate of colonization increases.
Keywords: Biofouling, Algae, Mortar, intrinsic characteristics

1. INTRODUCTION

In France, rendering is a common construction technique for masonry walls. Each year, 150 million m^2 of facade are coated with industrial mortars, corresponding to 3.5 million tons of mortars. Fifty-five percents are dedicated to new constructions and the remainder to renovation [1]. Exposed to weathering and affected by rain, wind, sunlight, CO_2, freezing/thawing cycles, and salt crystallization, rendered surfaces are progressively and inevitably subjected to biological colonization [2,3].

The color of the biofilm formed on the facades of buildings is mainly green, black or red stains, depending on the biological species. The main biological organisms are green algae and cyanobacteria, which pave the way for the implantation and growth of lichens, followed by bryophytes, pteridophytes, and finally spermatophytes [4-8]. Stains affect clearly the aesthetics of the façade and represent a significant economic loss, due to the costs of maintenance and repair.

The biological colonization of buildings surfaces depends on environment and substrate [7-9]. Crispim et al. [10] have shown that the composition and predominant phototroph species (cyanobacteria or algae) depend on the local climate (temperate or tropical). However, the most significant environmental factor controlling algal growth on building facades relates to the micro-climate (e.g., moisture, light) [11]. If moisture is high enough and if lighting and temperature conditions are suitable, colonization of the surface of new buildings can occur very quickly [4].

According to the substrate characteristics, physical (roughness, porosity) and chemical (mineral

composition, surface pH) contribute to biological colonization [6,7,12]. All of these parameters are included in the term "bioreceptivity" defined by Guillitte [13]. Among them, the roughness seems to be the most important factor [4,14]. Rough facades are more subjected to the biofouling than smooth ones [4,7,15,16]. The total porosity and pore size distribution are factors to take into account. They influence the absorption and retention of water by the material and the capillary rise on part of walls in contact with the soil [17-20].

The colonization is known to be much slower on freshly built concrete or cement-based surfaces, where the initial pH is higher than 11 [5]. With time, the combined action of water and atmospheric carbonation leads to a progressive decrease in pH of the building surface, which becomes low enough (about 9) to allow algal growth [9].

Identifying and quantifying the role of different intrinsic parameters of material on algal growth remain thus essential. In this study, the susceptibility of mortar to algal fouling was evaluated regarding the role of porosity, roughness, and surface pH by means of an accelerated test.

2. MATERIALS AND METHODS

2.1 Materials

The samples were made up of Portland cement CEM I 52.5 N (Holcim), siliceous sand (Sibelco DU 0.1/0.35), and calcareous filler (Omya) according to the proportions reported in Table 1. The water to cement ratio (w/c wt/wt) was fixed to 0.5. In order to obtain a more porous mortar, the w/c ratio was increased to 1 and hydroxylethyl methyl cellulose (HEMC) was added to thicken the mortar and to avoid segregation.

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

[a] in addition to dry mixture (cement, sand and filler)

The fresh mortar was cast into 50 × 50 × 1 cm expanded polystyrene moulds and stored at 21 ± 1°C and 95 ± 5% relative humidity during 28 days for the preparation of uncarbonated mortars. The plates were then cut into 20 × 8 × 1 cm samples. Carbonated samples were stored for only 7 days before cutting and carbonation step. The carbonation of the samples was achieved in a chamber under pure CO_2, at 21 ± 1°C, and 65 ± 5% relative humidity for 36 days. For each composition, three finishing methods were used during the setting. One method involved smoothing the surface of the samples with a ruler. The two others consist in scratching the surface with two different roughness sponges.

2.2 Characterization of Materials

The total porosity of materials was characterized using mercury intrusion porosimetry (Micromeritics Autopore IV 9400). The results reported represent the average of values obtained for three samples that were dried beforehand by acetone.

The surface roughness was measured using a CHR-150-L profilometer. The roughness was evaluated by the arithmetic average of the height Ra [21].

The pH of surface mortar was measured by a surface electrode (WTW Sentix Sur). To ensure contact between the substrate and the pH-electrode, the mortar surface was moistened.

2.3 Origin and Culture of the Algae

The algae used for this study was the filamentous green algae *Klebsormidium flaccidum* provided by the Muséum National d'Histoire Naturelle (Paris, France). This species was selected because of its representativeness. The strain was grown in a batch culture, with 400 mL of Bold's basal medium (BBM) [8]. The light was provided by two neon lamps with a 12h/12h light/dark photoperiod.

2.4 Accelerated Biofouling Test

The experimental device consisted of a 100 × 50 × 50 cm closed glass chamber placed in a dark room. In this chamber, 50 L of sterilized BBM and a controlled amount of the algae K. flaccidum were introduced. The initial algae concentration was fixed to 4 $mg.L^{-1}$ of dry mass for every test. The suspension was maintained at 24°C during all the test by means of a thermo-regulator.

Two rows of samples were placed back-to-back on a stainless-steel support inclined at 45°. Each row of samples was equipped with a sprinkling system (stainless-steel tube and pumps) which allowed to an algae suspension to sink on the top of each sample. The sprinkling period took place for 90 min every 12 h. The amount of suspension received by each sample during one cycle was around 26 ± 2 $l.h^{-1}$. The system constituted a closed circuit.

The light was provided by two neon lamps for 12 h/day and was set to start with the beginning of a sprinkling cycle.

In each test, 18 samples were placed in the chamber. Each formulation was tested in triplicate. Carbonated and uncarbonated samples were tested separately.

2.5 Evaluation of Biofouling

Image analysis was used in order to evaluate the biofouling. The surface of each sample has been digitized daily, using an office scanner. The numerical color image was converted in the YIQ color space [22] to improve the detection of algae on the surface of the cementitious samples. The Q channel was used to quantify the colonized surface by thresholding and segmentation. The colonization rate was given by the ratio of colonized area to the total surface. An example of image analysis carried out on a carbonated specimen prepared with a w/c of 0.5 and after 17 days of biofouling test is shown on Fig. 1.

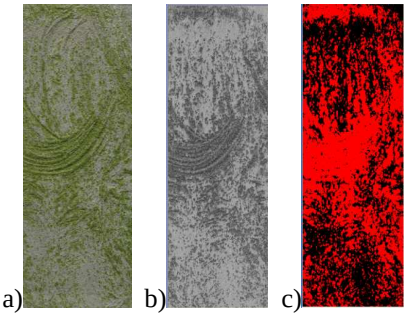

Fig.1 Example of image analysis of a specimen after 17 days of test
a) Original picture obtained by the scanner;
b) Q band after the conversion of image a) into YIQ color space;
c) Image obtained from image b) by thresholding and segmentation.

Fig. 1a corresponds to the original image obtained by digitization. Fig. 1b represents the Q band of the Fig. 1a after conversion into YIQ color space. Fig. 1c illustrates the segmentation of the image (red and black pixels correspond respectively to the fouled and un-fouled areas).

The latency time (t_l) was defined as the time corresponding to a colonization rate of 0.5 %.

3. RESULTS

3.1 Characterization of Materials

All the intrinsic characteristics of mortars are summarized in the Table 2. By using the two proposed w/c ratio and the HEMC for w/c=1, two levels of porosity were obtained. For w/c=0.5, the total porosity of samples ranges from 10 to 16%. As expected, the total porosity increases up to 32 and 37% for the w/c=1 and the HEMC.

The carbonation of the mortars was realized in the entire thickness. This was checked by the discoloration of a phenolphthalein solution deposing on the fracture surface of the sample. Concerning the surface of the sample, two levels of pH were obtained: 11 and 9 for uncarbonated and carbonated mortars, respectively. Moreover, the carbonation, by the crystallization of $CaCO_3$, leads to decrease the total porosity. Indeed, the carbonated samples are lightly less porous than their uncarbonated homologues.

By using the finishing method previously described, three levels of surface roughness were obtained. Despite of high standard deviation, the highest roughness (R3) was always four to six times higher than the lowest roughness (R1). The intermediate roughness (R2) presented asperities of the same order of magnitude as the radius of used sand.

Table 2 Intrinsic Characteristics of Mortars

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

The Fig. 2 illustrates the algae fouling of specimens at different test durations. The colonization by *K. flaccidum* produced a dense velvety mats formed by many entangled filaments, as noted by Rindi et al. [23]. The fouling of the samples surface was initiated by small spots of algae clinging to the surface. Most of time, the spots appeared at specific locations of the surface such as air bubbles or asperities. The extension of the biofouling resulted in the growth of the first spots and in the adhesion of new ones.

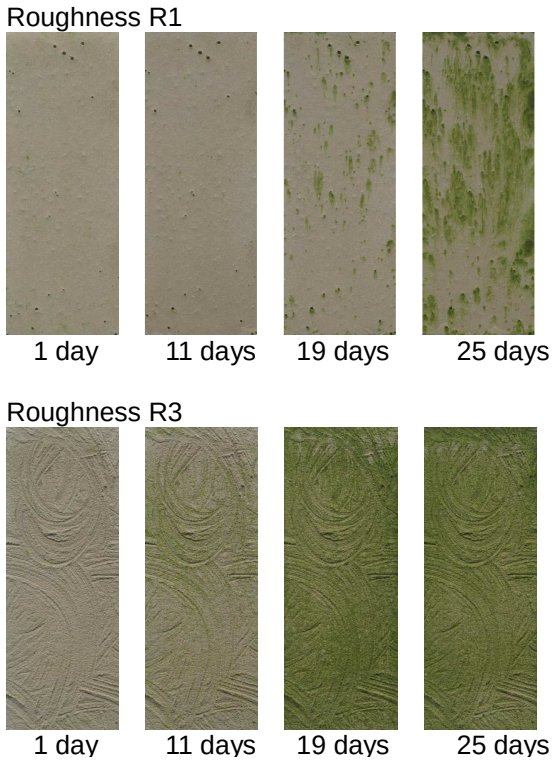

Fig. 2 Colonization kinetics by algae of carbonated mortars (w/c=1, roughness R1 and R3)

3.2 Influence of porosity

Fig.3 shows the influence of the w/c ratio on the colonization rate of the smoothest mortars. The colonization curves are all S-shaped. For carbonated samples, the colonization is nearly identical for both w/c ratios. The colonization starts 10 days after the beginning of the experimentation and the half of the surface is covered after 24 days. After 32 days, the mortars are completely covered by algae. For the uncarbonated mortars, a minor difference in the colonization rate occurred between formulations. The colonization of the mortars made with a w/c=1 seems to be slightly faster than that obtained with a w/c=0.5. The samples with the highest w/c, present surface defects (air bubbles), that may favor the algae attachment.

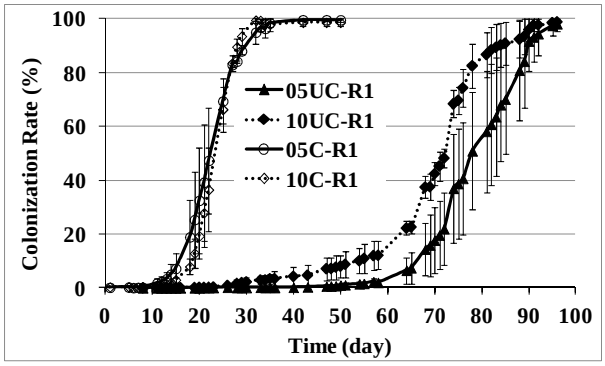

Fig.3 Influence of w/c ratio (0.5 and 1) on the colonization rate for uncarbonated (UC) and carbonated (C) mortars (roughness R1)

Despite of a significant difference in porosity, the colonization of mortars by algae is similar. This result can be explained by the permanent water saturation of mortars in our conditions. Indeed, the relative humidity in the chamber remained always around 100% during the darkness period and around 80% in the daytime.

3.3 Influence of roughness

Fig. 4 represents the effect of the roughness on the colonization rate for uncarbonated (UC) and carbonated (C) mortars (w/c=1). The slopes of the curves show that the rate of colonization of the smoothest surface was lower than that of the roughest one. After 32 days, the entire surface of the smoothest carbonated mortar (R1) was covered, while it took only 27 days for the carbonated mortar of intermediate roughness (R2) and 25 days for the roughest carbonated mortar (R3).

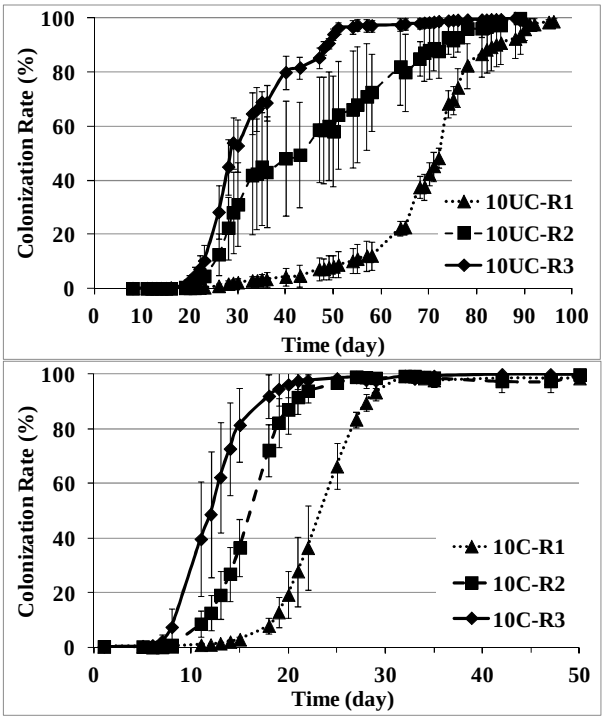

Fig. 4 Influence of roughness on the colonization rate for uncarbonated (UC) and carbonated (C) mortars (w/c=1)

Roughness is an important parameter that favored colonization of cementitious materials by algae. This result is consistent with those of previous studies [18-20]. Indeed, the roughness provides numerous asperities that promote the attachment of algae dispersed by runoff. For filamentous algae with smooth and thin-walled cells, such as *K. flaccidum* [23], this effect is pronounced. In contrast, for unicellular algae with thick walls, Guillitte and Dreesen [24] suggested a preference for smooth surfaces. The attachment is realized by a "suction-cup" phenomenon.

3.4 Influence of surface pH

The effect of carbonation, and thus surface pH, is shown on fig. 5 (mortars of roughness R1 and R3, w/c=1). The decrease in surface pH, by the means of carbonation, favors the biofouling of mortars. Indeed, the latency time and the rate of colonization (slope of

the curve) are strongly decreased by the carbonation. For example, t_l and the complete colonization of roughness R1 samples are respectively equal to 27 and 95 days for uncarbonated samples against 10 and 35 days for carbonated ones. The slope of the curves indicates a colonization rate of carbonated mortars significantly higher than that of uncarbonated ones. Similar results were observed by Shirakawa et al. [25] on the colonization of mortars by fungi. Indeed, he showed that pH values up to 9 allowed the colonization of mortars by *C. sphaerospermum*, while pH higher than 10 inhibited the fungal growth. The inhibition effect of high surface pH for algal colonization on stone was also underlined by Prieto and Silva [18]. Unlike the roughness, which impacted upon the ability of algae to physically cling to the surface, the carbonation affected the algal metabolism. Indeed, the alga Klebsormidium is well known as an acidophilic microorganism [26-28]. In alkaline media, ultrastructural changes in cells and a high proportion of dead cells were described by Škaloud [29].

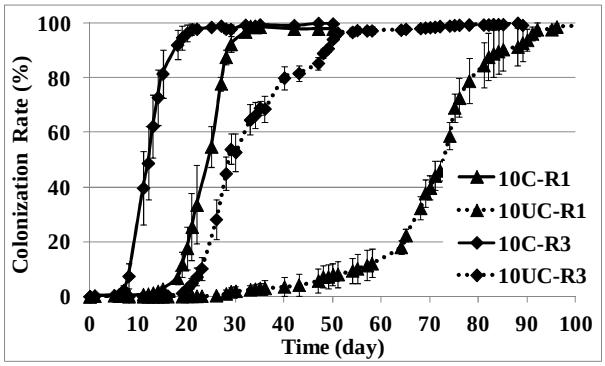

Fig. 5 Influence of carbonation on the colonization rate (mortars with roughness R1 and R3 and w/c=1) (solid line: pH=9; dotted lines: pH=11)

In order to conclude on the effect of pH, the concentration of algae into the suspension was followed by fluorescence. However, the algal concentration is quite similar for the experiments carried out with carbonated and uncarbonated samples. It means that the alkaline surface constituted extreme and unfavorable conditions for *K. flaccidum*.

3.5 Influence of intrinsic characteristics of mortars on the latency time

The Fig. 5 shows the influence of the roughness (R_a) and of the carbonation state of mortars on the latency time. For carbonated mortars, the latency time seems to slightly decrease with the roughness. However, for uncarbonated samples, the latency time is strongly dependant of the roughness. For example, t_l is equal to 18 days for R_a=123µm against 27 to 44 days for R_a=29µm.

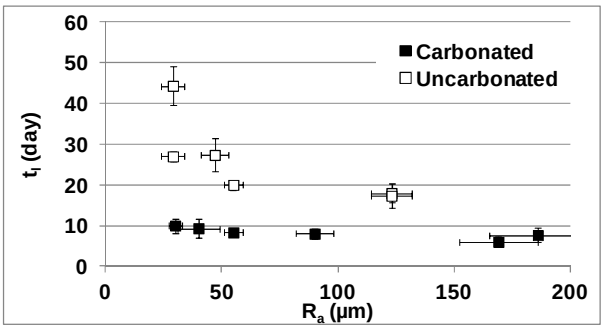

Fig. 5 Influence of roughness (R_a) and of carbonation state of mortars on the latency time

Finally, it is clear that the latency time is always higher for uncarbonated samples than for carbonated ones, and thus, strongly increases with the pH.

5. CONCLUSIONS

The test chamber simulating water runoff is adequate to study the relationship among intrinsic characteristics of materials in relation to biological fouling.

The role of porosity was not highlighted in this study because of the test conditions.

The laboratory test confirms the implication of roughness on the biofouling rate. When a lot of anchorage points are offered, the adherence of algae is promoted.

The other decisive parameter is the surface pH. Indeed the decrease in pH, by the means of carbonation, promotes algal development significantly. Thus the latency time is shortened and the rate of colonization increases.

Extrapolation of the results obtained under accelerated laboratory conditions to the real condition needs to be ascertained. The next investigative step is to extrapolate these results to real-world and natural conditions.

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