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AN *IN-SITU* AND LABORATORY STUDY OF THE EFFECT OF THE INTRINSIC PROPERTIES OF MORTARS ON THEIR POTENTIAL BIORECEPTIVITY

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Abstract

This study aims to clarify the effect of mortar intrinsic properties (porosity, roughness and carbonation level) on its ability to biofouling. Two scales experimental tests, an accelerated fouling in laboratory and a natural fouling in the real-world, were set-up. The first one was conducted in a closed device allowing a periodic sprinkling of an algal suspension on the samples surface. The outdoor test samples were exposed in a park at Grenoble (France). The colonization rate of the sample surface was evaluated by image analysis. The results show that the impact of each intrinsic parameter is quite different as function of the test. The porosity has no influence on the algal colonization of the samples exposed in indoor whereas a high porosity seems to increase slightly the bioreceptivity of ones exposed outdoor. The roughness, in both tests, promoted the microorganisms attachment and so their colonization. However, the discrimination of roughness grades is higher in the laboratory test than in the *in-situ* one. The surface pH significantly influences on the accelerated biofouling but not on the *in-situ* one. These dissimilarities result from the difference in experimental configurations of the two tests. Thus the laboratory test should be adjusted to be more suitable and to allow an extrapolation of results.

1 INTRODUCTION

Mortar and concrete are common material for building envelope. After a certain time, buildings are usually subjected to the colonization by microorganisms. Among the involved microorganisms, the chlorophytes (green algae) and the cyanophytes (blue-green algae) are the main and the initial colonizers [1-4]. This biological colonization induces an esthetical problem and can in later stage, degrades the building-materials by physical or chemical mechanisms [5].

The biofilm composition and the colonization rate depend on different parameters such as the material properties, the building architecture and the global and local environment around the building. In general, the conditions that favor the water retention and the access of material surface to the biological propagules, promote the implantation and the growth of microorganisms. For example, in rainy regions and during heavy precipitation season, the facades represent a higher susceptibility to biofouling [6]. The north-facing facades which are wetter and less sunny, get colonize faster [4,6]. Several authors notify the permanent biofouling observed at the foot of walls, the junction of different coatings and the overhanging elements (cornices, moldings, balconies, etc. ...) [4,7]. Furthermore, the humid environments near a lake, a river, trees and shrubs, are favorable to the appearance of stains on the walls.

The intrinsic properties influencing on the susceptibility of material to microorganisms colonization is defined as bioreceptivity [8]. It can be divided into physical properties (porosity, roughness, hydrodynamic properties...) and chemical ones (chemical composition, pH surface...). Several studies have investigated the role of material characteristics since the last thirty years [4,9]. However, most of them focused on laboratory scale experiments. The conditions of these tests (illumination, humidity, inoculation...) were chosen in order to accelerate the biological colonization process on the sample surface. Few researches were performed to examine what happens in the real world, i.e. at field-scale which requires many years. So, correlations between these two experiment scales are scarce. Furthermore, to accelerate the laboratory test, the cementitious material is often aged by carbonation and/or leaching process. However, the effect of the accelerated aging is rarely evaluated.

This work aimed to study the influence of intrinsic characteristics of mortar (porosity, roughness, carbonation state) on its biofouling. Two test scales are conducted: an accelerated laboratory test and a test by exposition of samples to natural conditions (*in-situ*).

2 MATERIALS AND METHODS

2.1 Preparation and characterization of materials

The investigated materials are mortars composed of 30 % Portland cement (CEM I 52.5), 65 % siliceous sand (0.1/0.35) and 5 % calcareous filler. To this dry mixture, 0.27 % cellulose ether (Hydroxylethyl Methyl Cellulose) was added. In order to obtain different porosities without changing the mortar formulation, two water/cement (*w/c*) ratios, 1 and 1.2, were used.

The fresh mortar was casted into 50 x 50 x 1 cm³ expanded polystyrene molds and stored at 20 ± 1 °C and 95 ± 5 % of relative humidity. After 28 days of curing, the mortar was cut into 20 x 8 x 1 cm³ and 30 x 20 x 1 cm³ specimens for accelerated tests and *in-situ* tests, respectively. To investigate the effect of carbonation, the mortars were cut in same size specimens, after only 7 days of storage, and were then exposed to an accelerated carbonation until reaching a surface pH of about 9. The carbonation was performed under pure CO₂ at 20 ± 1 °C and 65 ± 5 % of relative humidity.

Different surface roughnesses were achieved by three finishing methods of mortar during its setting. The first consisted in smoothing the surface of fresh mortar by a ruler and the two others in scratching the surface of mortars during setting with two sponges of different roughness.

The porosity was determined by mercury intrusion porosimetry. For each mortar, three samples were measured after prior drying by acetone.

The surface pH was measured thanks to a surface pH electrode (Sentix Sur).

The roughness of the mortar was evaluated by means of an optical profilometer and characterized by the arithmetic average of the height (R_a) [10].

2.2 Biofouling tests

2.2.1 Laboratory scale tests

The laboratory fouling test was performed by means of a closed device containing an algal suspension. The microalga *Klebsormidium flaccidum* was selected because of its representativeness [4,11-13]. Furthermore, *K. flaccidum* is easy to cultivate.

The initial concentration of algal suspension was fixed to 4 mg/ml (of dry masse) and 50 L of this suspension was introduced into the test chamber. Thanks to a pump system, the sample surfaces were sprinkled for 90 min every 12 h by this suspension. The light was provided by two neon lamps. The photoperiod was fixed to 12 h light/12 h dark. The details of the experimental approach were described in previous papers [14,15].

In laboratory experiments, specimens were tested in triplicate for each material. Carbonated and uncarbonated samples were tested separately.

2.2.2 Field scale tests

In the field-scale experiments, the mortar samples were placed on a stainless steel frame in a private green park close to Grenoble, France. In order to favor the biological colonization, the samples were placed near trees, facing to north and inclined with an angle of 45 °. Furthermore, to avoid splashing during rainy periods, the specimens were arranged in two rows of which the first was positioned at 1 m above the ground. The contamination between specimens was also avoided.

For each material, three specimens were tested. The mortars with a w/c ratio of 1 were studied at carbonated and uncarbonated states, with the three roughnesses. For the samples with a w/c ratio equal to 1.2, only the carbonated mortars with the two highest roughnesses were examined.

2.3 Biofouling evaluation

The fouled area was determined by means of image analysis. Each specimen was digitalized thanks to an office scanner every weekday and at least every two months for bench-scale and field-scale experiments, respectively. The RGB color space images obtained were transformed thanks to Aphelion software into YIQ color space to improve the detection of fouled surface. The biological coverage was well detected on the grey level image of the Q-channel by thresholding. The colonization rate was calculated by the ratio of the colonized area to the total area. More details about this method can be found in [14].

3 RESULTS AND DISCUSSION

3.1 Material characteristics

The characteristics of mortars are given Table 1. For both uncarbonated and carbonated mortars, the increase in the w/c ratio induced a rise in the total porosity. Indeed, the porosity increases from 37 % to 39 % for uncarbonated mortars and from 32 % and 36 % for carbonated mortars when w/c increases from 1 to 1.2. The accelerated carbonation leads to a reduction of surface pH from 11 for uncarbonated mortars to 9 for carbonated. Three levels of roughness were obtained whatever the carbonation state and the w/c ratio. The roughnesses are around 30 μm , 50 μm and higher than 70 μm for the lowest, the intermediate and the highest roughnesses, respectively.

Table 1: Characteristics of mortars

Sample	w/c ratio	Porosity (%)	Surface pH	Roughness R_a (μm)
1UC-R1	1	37.2 \pm 0	11.0 \pm 0.4	29 \pm 5
1UC-R2				47 \pm 6
1UC-R3				70 \pm 8
1.2UC-R1	1.2	38.9 \pm 0.3	11.0 \pm 0.3	29 \pm 5
1.2UC-R2				47 \pm 6
1.2UC-R3				123 \pm 9
1C-R1	1	32.1 \pm 1.9	9.0 \pm 0.1	29 \pm 5
1C-R2				47 \pm 6
1C-R3				138 \pm 15
1.2C-R1	1.2	36.2 \pm 0.1	8.9 \pm 0.1	32 \pm 4
1.2C-R2				47 \pm 6
1.2C-R3				145 \pm 18

3.2 Colonization of cementitious material surfaces by microorganisms

An illustration of the algal colonization in both tests is shown Figure 1.

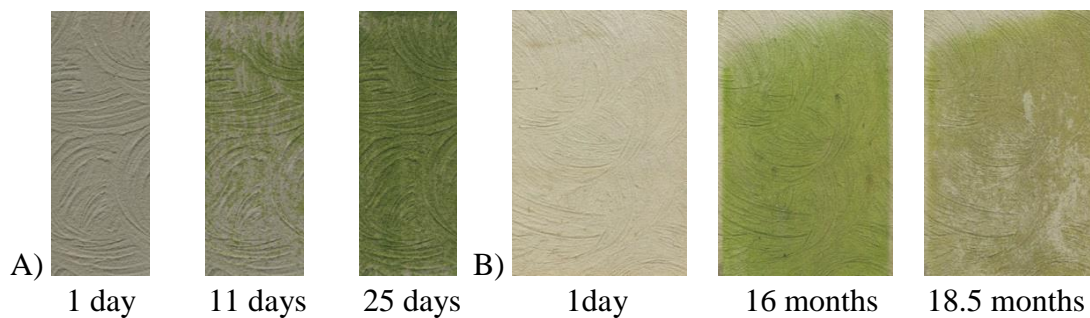


Figure 1: Illustration of algal colonization over time in laboratory test (A) and *in-situ* test (B), for 1.2C-R3.

In the accelerated test, the growth of *Klebsormidium flaccidum* generates a dense and velvety mat on samples as illustrated in Figure 1. The first algal spots appear at privileged sites of the surface, such as air bubble holes or asperities formed by the roughness. The extension of fouling results from the growth of the first spots and the adhesion of new ones. On the lowest roughness samples (R1 and R2), the algal growth forms streaks due to the

suspension flow. This fouling form is usual on the building facades. For the roughest mortars (R3), the fouling follows the surface asperities.

In the field-scale tests, the samples were exposed to natural conditions from summer 2009 (26th of June). During almost one year, no visible biological colonization was observed on the samples. The first settlement appeared on the mortars 1.2C-R3. The main colonizer was attributed to green algae. Indeed, the biofouling of all the *in-situ* samples was green, which is the characteristic color of green algae. The colonization rate of 1.2C-R3 mortars is shown in Figure 2B. The curve can be divided into four steps: a latency step, a growth step, a stagnation step and a decrease step. The latency time was about 11.6 months. Furthermore, the biological growth accelerated between 12 and 16 months of exposure corresponding to the autumn period (from August to November). In fact, the propagules of microorganisms might be already settled on the surface since the spring. Then, mild temperatures coupled with strong light and heavy rainfall during this autumn favored the biological growth. After a short period of stagnation, from 16 to 18 months of exposure, the colonization rate decreased. In fact, the microorganisms detached from the surface. Cell aging and cell death due to the low ambient temperatures, the low light densities, the snow and the effect of outdoor conditions themselves, disrupted the anchoring between the microorganisms and the sample surface.

3.3 Effect of intrinsic characteristics of mortar

3.3.1 Porosity

The effect of the w/c ratio (1 and 1.2), and thus the porosity, on the biofouling rate of carbonated mortars is shown in Figure 2.

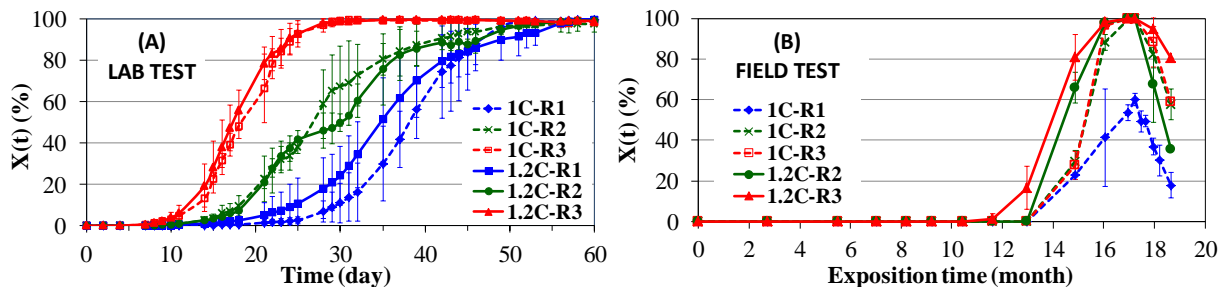


Figure 2: Effect of porosity (w/c ratio) and roughness on the colonization rate for carbonated mortars in laboratory (A) and field tests (B).

In the bench-scale experiment (Figure 2A), the algal colonization curve is of sigmoid type, characterized by three steps: a latency step, an exponential growth step and a stagnation step. For each roughness (R1, R2 and R3), the colonization rate is very close regardless the w/c ratio, especially, the roughest mortars (R3) where the two curves almost coincide. The algal colonization appears after 6 testing days and the entire surface is colonized after 30 days. For the two others roughnesses (R1, R2), because of experimental standard deviation, the colonization was considered identical whatever the w/c ratio.

In this laboratory test, porosity does not influence on the colonization rate, although the literature highlights an increase in bioreceptivity of porous mortars due to their water uptake and water retention behavior. This contradiction was allocated to the experimental protocol rather than to the modest difference in porosity between the two formulations. Indeed, relative

humidity in the test chamber was permanently very high (i.e. ranging from 80 to 100 %). In these conditions, water content into the samples was always abundant and thus no longer a limiting factor for the biological development. This observation was confirmed by previous studies regarding mortars made with w/c ratios equal to 0.5 and 1 [14,15]. Despite of the considerable difference in porosity, these mortars exhibited identical bioreceptivity.

Unlike in the accelerated test, the w/c ratio and thus the porosity exhibit an influence on the biofouling of *in-situ* samples (Figure 2B). Indeed, the visible microorganisms develops faster on the more porous mortars ($w/c = 1.2$). Between 11 and 13 months of exposure, while the biofouling appeared on the 1.2C-R3 mortars, no biological colonization was observed on the 1C-R3 ones. The following measurement, realized two months later, showed a colonization rate of 80 % for 1.2C mortars against only 30 % for 1C mortars. For the roughness R2, the effect of the w/c ratio was intermediate. One month later, only the more porous mortar was totally colonized. The results are in agreement with the literature since the mortars made with the highest w/c ratio were the most porous and exhibited a greater bioreceptivity [11,16,17].

3.3.2 Roughness

In the accelerated test, the biofouling rate was obviously influenced by the surface roughness of samples and the effect of the three levels of roughness was quite distinct (Figure 2A). Indeed, the colonization rate increases, while the latency time decreases with the roughness. The latency time was around 18, 9 and 6 days of testing, and the complete colonization was achieved in 56, 51 and 30 days for roughnesses R1, R2 and R3, respectively. These results are in agreement with previous studies [14,17]. By providing asperities, the roughness favors the physical anchorage of microorganisms provided by the suspension. Consequently, rougher is the surface, earlier the biofouling begins and faster the colonization is.

The roughness impacted also the biofouling of mortars exposed in the real-world by increasing the colonization kinetics (Figure 2B). However, only two grades of roughness were observed. Indeed, no difference in bioreceptivity to visible photosynthetic organisms was noticed for roughnesses R2 and R3. Conversely, roughness R1 exhibited a lower colonization rate. Therefore, the biofouling is less affected by the roughness in the field-scale test than in the bench-scale test that could be explained by a much less intense natural inoculation. Furthermore, contrary to the bench-scale test, for a given w/c ratio, the latency time was quite similar whatever the roughness. This could be the result of the mutual impact of the season, the external relative humidity and temperature in natural conditions. Thus the effect of roughness on the latency time is hid.

3.3.3 Accelerated carbonation

Figure 3 illustrates the evolution of the colonization rate for carbonated and uncarbonated mortars made with a w/c ratio of 1, for the lowest and the highest roughnesses (R1 and R3).

In the accelerated fouling test, the carbonation appears as the most discriminating parameter influencing the bioreceptivity of material by significantly shortening the latency time and accelerating the kinetics of algal colonization. Indeed, thanks to carbonation, the latency time decreases from 46 to 18 days and from 26 to 7 days for the roughnesses R1 and R3, respectively and the necessary time to reach the complete colonization is reduced (51 days for 1UC-R3 against 30 days for 1C-R3). The total colonization of 1UC-R1 was not achieved because of a precocious stop of testing. Nevertheless, the slope of the curve was greater for carbonated mortars than for uncarbonated ones.

Unlike the effect of roughness which favors the ability of algae to physically cling to the surface, the carbonation affects the biological metabolism. Carbonation, by decreasing the surface pH and thus the pH of the algal suspension, creates less alkaline environment and thus less stressful conditions for algae *K. flaccidum* [14,15]. Consequently, carbonated mortars are highly receptive to colonization by microorganisms.

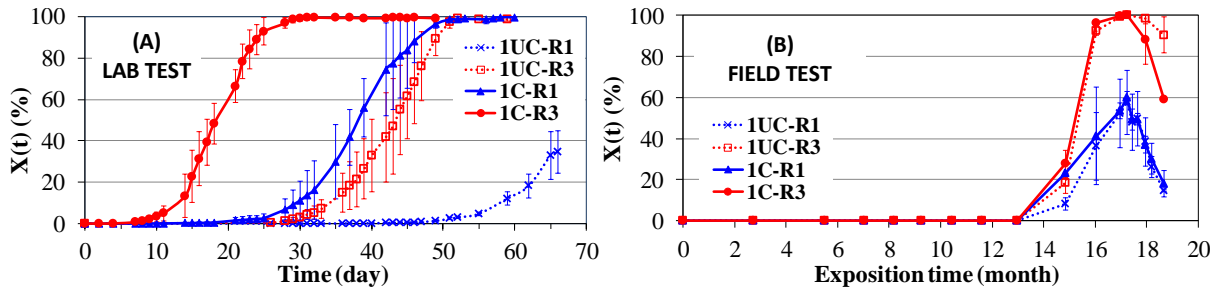


Figure 3: Effect of accelerated carbonation on the colonization rate for mortars made with a w/c ratio of 1, with roughness R1 and R3, in laboratory (A) and field tests (B).

In the field-scale test, the accelerated carbonation did not result in different growth kinetics, since the biological growth of phototrophic microorganisms evolved quite identically (Figure 3B). Indeed, whatever carbonation state of mortars, the latency time was about 13 months for all the formulations, and 4 months later, the total colonization was reached for all rough samples (R3) and 60 % for all smoothest samples (R1).

The favorable climatic conditions and sufficient inoculation were perhaps not met at the beginning of testing. Indeed, the samples were exposed through the summer, thus, spring, the most favorable season for spreading and growth of microorganisms, was past. Hence, the microbial colonization of carbonated samples was not initiated despite a favorable surface pH. After one year of exposure, the mortars were aged and weathered by leaching and natural carbonation. Consequently, the same surface pH ($\text{pH} \approx 8$) was measured for all the samples whatever their initial carbonation state. The bioreceptivity of mortars was thus identical when favorable conditions to the biological development were satisfied. Therefore, the influence of the initial surface pH on biofouling is completely inhibited.

4 CONCLUSIONS

By both laboratory and *in-situ* tests, the effect of the intrinsic parameters of mortars on their biofouling by photosynthetic organisms has been investigated.

The impact of porosity on biofouling of mortars was different for two experimental test scales. This parameter did not affect the colonization rate in laboratory test due to the experimental conditions. However, although more experiments of verification still required for the field-scale test, high porosity seems to favor the biological colonization.

For the both test scales, the influence of the roughness is evidenced. A rough surface enhances the biological attachment. The discrimination of roughness grades was better in the accelerated tests than in the field-scale ones, which could be explained by an intense inoculation of the accelerated test against a natural inoculation in the *in-situ* test.

The accelerated carbonation was considered as a decisive parameter in the laboratory test, by significantly promoting the algal growth and by accelerating the colonization rate. While

in natural conditions, due to numerous uncontrolled and random environmental factors, the effect of the carbonation was not observed.

The divergence of results between the laboratory and field-scale tests prevents to correlate the two experimental scales. These dissimilarities could be remediated by improving the experimental protocol such as a decrease in the inoculation intensity, a drying phase of samples between two sprinkling cycles in the accelerated test or a reasonable choice of the beginning of sample exposure to natural environment.

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