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Bioreceptivity evaluation of cementitious materials designed to stimulate biological growth

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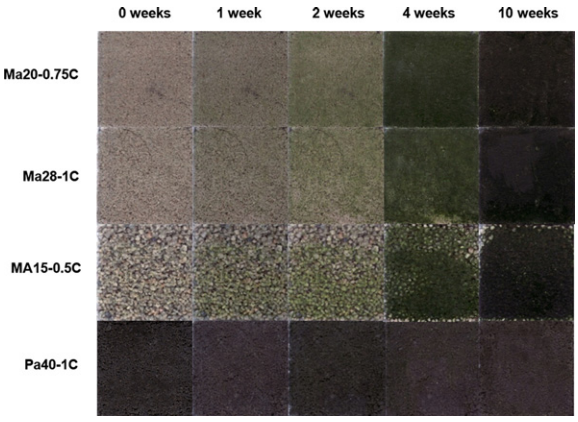
HIGHLIGHTS

- Magnesium phosphate cement suitability to stimulate colonisation is evaluated.
- Quantification of algal biomass by PAM-fluorometry was carried out.
- Fouling intensity parameter is suitable until complete coverage of specimens.
- Magnesium phosphate cement based mortars showed higher bioreceptivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Ordinary Portland cement (OPC), the most used binder in construction, presents some disadvantages in terms of pollution (CO_2 emissions) and visual impact. For this reason, green roofs and façades have gain considerable attention in the last decade as a way to integrate nature in cities. These systems, however, suffer from high initial and maintenance costs. An alternative strategy to obtain green facades is the direct natural colonisation of the cementitious construction materials constituting the wall, a phenomenon governed by the bioreceptivity of such material. This work aims at assessing the suitability of magnesium phosphate cement (MPC) materials to allow a rapid natural colonisation taking carbonated OPC samples as a reference material. For that, the aggregate size, the w/c ratio and the amount of cement paste of mortars made of both binders were modified. The assessment of the different bioreceptivities was conducted by means of an accelerated algal fouling test.
MPC samples exhibited a faster fouling compared to OPC samples, which could be mainly attributed to the lower pH of the MPC binder. In addition to the binder, the fouling rate was governed by the roughness and the porosity of the material. MPC mortar with moderate porosity and roughness appears to be the most feasible material to be used for the development of green concrete walls.

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1. Introduction

Portland cement (OPC), the most used hydraulic binder in construction, presents advantages in terms of durability and heat storage capacity. However, its production implies environmental disadvantages concerning energy consumption and CO_2 emissions (production of approximately 1 kg of CO_2 per kg of OPC resulting in a 6–7% of the annual worldwide CO_2 emissions) (Formosa, 2012). Moreover, the use of this material has an aesthetic impact mainly due to the predominant grey colours. Therefore, interest in environmentally friendly hydraulic binders like magnesium-phosphate cements (MPCs) is currently increasing.

Regarding energy consumption, 4 GJ of energy are necessary to manufacture 1 ton of Portland cement (Malhotra, 1999; Mehta, 1999). However, magnesium oxide (being the raw material of magnesium phosphate cements) is readily obtained from calcining magnesite. This process of decomposition occurs at temperatures around 400 °C, which is much lower than the temperature required to decompose limestone and to form alite in Portland clinker production. Consequently, the production of MPC requires less energy than the production of OPC (Phair, 2006), which is around 1.45 GJ/ton for the MPC dosage used in the current study. The neutral or slightly acidic pH of MPC allows the creation of concrete structures that can sustain or stimulate biological growth (Manso et al., 2014).

Green roofs and facades gained considerable attention in the last decade. In addition to decreasing the heat build-up in cities, resulting from insolation, they also have an aesthetic function, i.e. creating green areas in a predominantly grey urban environment (Pérez Luque, 2010; Ottelé et al., 2011). Nowadays, two major technologies are used to create vegetated wall surfaces: systems rooted in the ground and systems rooted in artificial substrates. The first group refers to green walls made of climber plants with or without supporting systems and is the most traditional technology. In contrast, the second group is characterized by its dependence on irrigation systems and addition of nutrients to the substrate. Different technologies such as planter boxes or hydroponic systems are enclosed within this group (Perini et al., 2011). Both systems, however, suffer from high initial and maintenance costs (Pérez Luque, 2010). Integration of the green coverage in the structure of the building could provide a means to minimize these costs.

So far, however, attempts to use concrete as a substrate for "green" biological growth were unsuccessful. This could be mainly attributed to the high pH of the cementitious material, OPC. Biological growth usually appears after carbonation of the concrete surface, a process that induces a decrease in the pH from 13 to around 9. However, biological growth observed is usually unsightly. The final pH and the heterogeneity of this material provide a fast support for cyanobacteria colonisation, which predominant colour is almost black.

The use of concrete as a substrate for biological growth thus requires modifications of its primary bioreceptivity, which was defined by Guillitte (1995) as "the totality of material properties that contribute to the establishment, anchorage and development of flora and/or fauna". One of the most important properties of the primary bioreceptivity of a substratum is the pH and consequently, the chemical and mineral composition (Wilimzig and Bock, 1996; Warscheid and Braams, 2000). Secondly, properties such as porosity and roughness will also define the nature of the colonisation due to the retention of organic matter, dust and raindrops and the provision of anchorage spots for the micro-organisms (Guillitte and Dreesen, 1995; Silva et al., 1997). However, there are other parameters affecting the bioreceptivity of a substratum (hygroscopicity, colour of the material, albedo, and so on) (Guillitte, 1995).

Therefore, producing cementitious materials that stimulate natural colonisation would improve current designs. Moreover, this would not only have an aesthetic advantage, but also ecological and environmental benefits are expected. As mentioned before, living wall systems (LWS) present advantages because of the presence of photosynthetic organisms. In this particular case, they are basically plants although most of these benefits are also reported for other photosynthetic organisms. These organisms use CO_2 and sun light energy for photosynthesis. Green walls based on plants reduce solar energy through shading and reduce heat flow through evaporative cooling, both translating into energy savings (Wong et al., 2010). When light is in excess, photosynthetic organisms reflect or transform into heat part of this energy. Depending on the amount and type of species present, these processes could be significant (Maxwell et al., 1994).

This study fits within the framework of the development of a multilayered concrete panel (Manso et al., 2013). This patented technology proposes an improvement of the bioreceptivity of cementitious materials to allow biological growth in one of its layers. For this purpose, evaluation of suitability of different primary bioreceptivities is required. The first layer consists of conventional concrete and is responsible for the structural function of the panel. Production of this layer would depend on project requirements. Subsequently, there is a second layer with the main function of protecting the first one. This layer has a waterproof capability and could also improve the adhesion between the first and the third layer. The third layer is the one with an improved bioreceptivity and the one related to the current study. Thanks to this improvement of bioreceptivity, rain water retention as well as stimulation of colonisation by living organisms will be achieved. Finally, the fourth and last layer would be a discontinuous one in order to allow different designs of the surface. Areas without this layer can retain water which allows organisms to colonise the surface. Exit of water is then redirected to these areas promoting better local conditions for colonising organisms.

Although biological growth on concrete surfaces is generally considered as pathological due to biodeterioration, many studies have also indicated its protective function (Ariño et al., 1995; Gaylarde and Gaylarde, 2005; De Muynck et al., 2009). In fact, same knowledge could be applied for both prevention as well as stimulation of biofouling. Therefore, modifying primary bioreceptivity physically and chemically opens up new possibilities. First, chemical alterations to reduce the pH will allow a greater variety of organisms to colonise the surface. Subsequently, physical changes will also provide optimal conditions for colonisation. Finally, the patented system will protect the structural component as well as play with the design in order to favour homogeneous colonisation of specific areas.

Numerous research groups have been investigating biofouling on building materials, and consequently, proposing different methodologies of evaluation. In this study, the modular accelerated algal fouling test developed by De Muynck et al. (2009) was implemented. Contrary to setups like the types proposed by other authors (Guillitte and Dreesen, 1995; Dubosc et al., 2001; Barberousse et al., 2007; Escadeillas et al., 2007), the modular setup by De Muynck et al. (2009) allowed the simultaneous evaluation of chemically different concrete mixtures. Consequently, contamination of the algal cultures by concrete leachate will be avoided.

The current study fits within the framework of the development of a multilayered concrete panel designed to stimulate biological growth. More specifically, the purpose of this research was to select a concrete composition that is most suitable to function as a non-structural element of the multi-layered panel. For this purpose, the bioreceptivity of concrete mixtures with different roughness and porosity was evaluated by means of an accelerated algal fouling test. Magnesium phosphate cement was selected as a promising binder for the non-structural element, because of its low pH. Fouling tests were also performed with OPC concrete mixtures, in order to allow a clear evaluation of the difference in fouling rate and behaviour between the two types of binders.

2. Materials and methods

2.1. Mortar specimens

With the aim of producing OPC and MPC specimens with different porosity and roughness, two different silica aggregate sizes were used (0/2 mm and 2/4 mm). The composition of the mortars is given in

Table 1. Furthermore, variations in water to cement (w/c) ratio and amount of cement paste were used. The water to cement ratios ranged between 0.3 and 0.6 and between 0.15 and 0.28 for OPC and MPC specimens, respectively, based on the Spanish Standard for OPC (UNE-EN 196-1:2005, 2005) and on the characterization previously carried out for OPC and MPC mortar bioreceptivities (Manso et al., 2013).

For the estimation of the amount of cement paste required for porous concretes, the method proposed in previous works (Klein, 2012; Klein et al., 2012) was used. The amount of cement paste obtained from Klein's formulas was fixed as the minimum amount of cement paste to join all the aggregate particles. In order to study the effect of a modified porosity, OPC mortar samples with a higher amount of cement paste were additionally prepared. However, although the method worked perfectly for OPC samples, the behaviour of MPC samples was completely different. Here, the amount of cement paste obtained from the method was used as the biggest amount of cement paste. Consequently, with the aim of having MPC specimens with different porosity, samples with a reduced amount of cement paste (in comparison with the amount obtained by the Klein formula) were additionally prepared.

OPC and MPC specimens were cast into $80 \times 80 \times 20$ mm^3 polyurethane elastomer moulds. The powder phase of the magnesium-phosphate cement consisted of a mixture of magnesium oxide (MgO) as the basic component, monoammonium phosphate ($NH_4H_2PO_4$) as the acidic component and sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), also known as borax, as a retardant. MPC specimens were demoulded after 1 h while OPC samples were demoulded after 24 h. Both types of specimens were allowed to cure at 22 ± 2 °C and 95 ± 5% relative humidity for 28 days even though this curing process is not required for MPC.

As the surface pH of the OPC concrete mixtures was too high to allow biological growth, samples were subjected to accelerated carbonation by storing them for 3 weeks in a container with 100% CO_2 (65 ± 5% RH and 1 atm).

The compressive strength was determined at 28 days by an automatic concrete compression machine (IBERTEST) according to UNE-EN 196-1:2005 (2005). The porosity was determined according to ASTM C642-13 (2013) to estimate the percentage of voids in the hardened specimens after carbonation.

Furthermore, the roughness was determined by means of a high precision laser beam (sensor ILD 1900-50 and optoNCDT 1800 interface, Micro-Epsilon Messtechnik GmbH, Germany) mounted on an automated laser measurement table developed at Ghent University (De Belie et al., 2004). From the measurements of the surface profile, the centre-line roughness (R_a) can be calculated according to the standard BS 1134:2010 (2010). For the determination of the roughness, 72 surface profiles were obtained from 3 replicates for each of the six compositions (four profiles per specimen with profile length 8 cm and 1.5 cm between each profile) (De Muynck et al., 2009). For each profile, after correction for the slope, three R_a-values were measured over separate parts of the profile (reference length: 40 mm). The R_a-values presented in Table 1 were calculated as the average of the 12 R_a-values obtained per specimen type.

Two methods for pH determination were carried out (immersion test and crushing with subsequent dispersion of the specimen in distilled water). The first method consists of placing the specimens inside a box filled with distilled water and keeping the boxes covered. Measurements of pH were carried out until stabilization. In contrast, the second method consists of crushing a piece of mortar and immersing the crushed material in distilled water at a liquid:solid ratio of 10:1. The resulting mixture was homogenized by means of a stirrer. The pH was determined by means of a pH-meter BASIC 20 (CRISON) after 1 h of mixing. Both methods differed to about 0.5 points of pH and results obtained with the first method are presented.

2.2. Accelerated algal fouling test

The algae specie used in this study was *Chlorella vulgaris* var. *viridis Chodat*. The strain was obtained from the culture collection of algae and protozoa (CCAP) from the Dunstaffnage Marine Laboratory (Scotland) (accession number CCAP 211/12). Batch cultures of these algae were grown under sterile conditions in Erlenmeyers containing 1 L of Walne medium with the addition of 0.2 mg of thiamin chlorhydrate and 0.01 mg of vitamin B12 (http://www.ccap.ac.uk/media/documents/Walnes.pdf). The Erlenmeyers were continuously exposed to light by means of Sylvania Grolux 30 W lamps on a KS 501 rotary shaker (IkaWerke, Germany) at 100 rpm. Air was provided by means of an Air plus 3 air pump. For the preparation of the medium, 2 mL of sterile concentrated Walne and 0.2 mL of vitamin solution were added to 1 L of autoclaved mineral water (Cristaline, natural spring water, Merignies, France). Each week, new batch cultures were grown by transferring 200 mL of the one week old culture to 1 L of fresh medium. The remaining culture solution was used to inoculate the water in the PET bottles used in the accelerated run-off test (De Muynck et al., 2009). The amount of cells per mL was determined by means of a Zeiss Axioskop II plus light microscope (Zeiss, Germany) and a counting chamber.

The accelerated algal fouling test was carried out by means of a modular water run-off test developed at the Magnel Laboratory for Concrete Research of Ghent University (De Muynck et al., 2009). The setup consisted of 6 stainless steel compartments placed with an inclination of 45°. Each compartment was equipped with a plastic sprinkling rail

Table 1
Specimens' composition.

Specimens	Composition	Compressive strength (MPa) (n = 6)	Porosity (%)	Roughness R_a (μm) (n = 12)	pH
Pa40-1C	Sand 0/2 mm a:c:w[a] = 4.41:1:0.4	15.83 ± 0.7	22.97	0.03 ± 0.00	≈9
Pa60-1.75C	Sand 0/2 mm a:c:w[a] = 3.22:1:0.6	46.81 ± 1.1	12.27	0.03 ± 0.01	
PA30-1C	Sand 2/4 mm a:c:w[a] = 3.8:1:0.3	27.52 ± 2.0	10.60	0.16 ± 0.02	
Ma20-0.75C	Sand 0/2 mm a:c:w[b] = 4.81:1:0.2	9.82 ± 0.1	18.20	0.04 ± 0.00	6.7
Ma28-1C	Sand 0/2 mm a:c:w[b] = 4.03:1:0.28	24.45 ± 1.4	2.47	0.06 ± 0.00	
MA15-0.5C	Sand 2/4 mm a:c:w[b] = 6.6:1:0.15	9.18 ± 0.8	13.15	0.15 ± 0.01	

$NH_4H_2PO_4$:MgO ratio = 1:1.75 and the addition of borax amounted to 6% by weight of the sum of $NH_4H_2PO_4$ and MgO weights; a:c:w is the ratio aggregates:cement:water; n is the number of measures. Labels of specimens make reference to the binder (P: OPC; M: MPC), aggregate size (a: 0/2 mm; A: 2/4 mm), w/c ratio (e.g.: 20 is 0.2 and 60 is 0.6), cement paste content (factor multiplying the minimum amount of cement paste needed to join all the aggregate particles, *C*, according to Klein et al. (2012)).

[a] CEM I 52.5R.

[b] Cement made by $NH_4H_2PO_4$, MgO and borax.

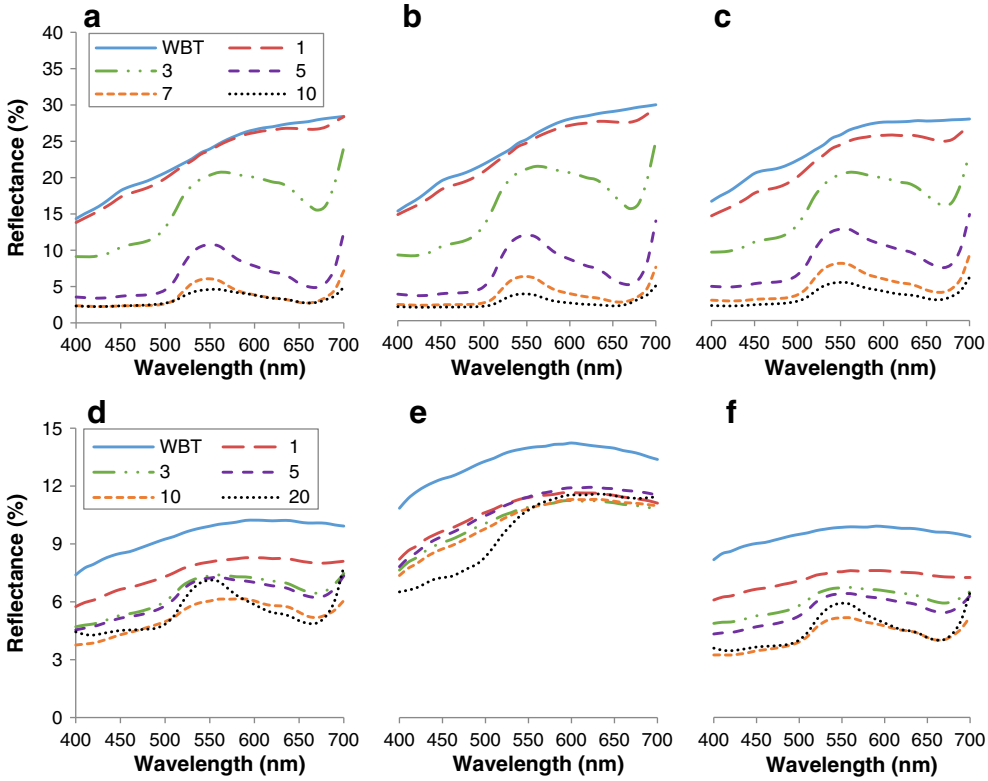

Fig. 1. Change in reflectance for different specimens in function of the number of weeks subjected to accelerated fouling; WBT: wet specimens before test. a) Ma20-0.75C, b) Ma28-1C, c) MA15-0.5C, d) Pa40-1C, e) Pa60-1.75C and f) PA30-1C.

(2 mm holes every cm) on top and a plastic gutter at the bottom. A transparent 2 L PET bottler below each compartment was used as a reservoir for the algal cultures. A Newair NW33 aquarium pump (200 L/h) was placed inside the PET bottle to circulate the algal cultures through a plastic tube connected to the sprinkling rail. Finally, water running down from specimens was collected by means of the gutter and a funnel covering the PET bottle (De Muynck et al., 2009). A total of 18 specimens were tested, corresponding to three replicates for each one of the six dosages. Specimens were placed in threes in a total of six independent compartments of the modular run-off test. Three of them were used to place OPC specimens and the other three to place MPC specimens to separate the specimens with different chemical composition. Then, one replicate per dosage was placed in a different compartment in order to avoid possible effects of variability among compartments.

The run-off period was set to start every 12 h and ran for 90 min. Furthermore, the setup was submitted to a 12 h day and night regime, which started simultaneously with the run-off periods (De Muynck et al., 2009). During the day regime, light was provided by means of Sylvania Grolux 30 W lamps. The temperature and relative humidity ranged between 22 °C (night)–25 °C (day) and 82% (day)–90% (night), respectively.

To prevent settling of the algae in the reservoirs and to provide O_2 and CO_2, the algal suspensions were continuously aerated by means of Air plus 3 air pumps. The mineral drinking water contained in the PET bottles was used for the preparation of the culture medium. The contents of each 2 L bottle were used to fill two PET bottles used as algal reservoirs (1 L per reservoir). Subsequently, 2 mL of concentrated Walne medium and 0.2 mL of vitamin solution were added to each reservoir. Algae from one week old cultures were inoculated in the reservoir at final concentrations of about 6.5×10^8 cells L^{-1}, corresponding to about 2.5 mg dry weight per litre. Every week, the contents of the reservoirs were replaced by new algal cultures, after cleaning of the reservoirs. Additionally, every two weeks, the reservoirs were replaced by new ones.

2.3. Evaluation and quantification of biofouling

Different tests were carried out in order to evaluate the visual aspect and degree of bioreceptivity of the samples. The specimens were studied weekly and in triplicate, immediately after the run-off period in order to have specimens in the same humidity condition. Furthermore, excess water on specimens' surface was removed with a paper towel.

First, colorimetric measurements were performed by means of an X-Rite SP60 colorimeter (X-Rite, USA) with an 8 mm aperture. Four measurements per specimen at fixed positions (on the four corners at 1 cm from the borders) were carried out. Before starting the accelerated algal fouling test, colorimetric measurements were taken at different humidity conditions of the specimens (from wet to dry). This procedure allowed examining the influence of humidity on the fouling evaluation parameters.

Characteristic pigments of *Chlorella vulgaris* are chlorophyll a, b and carotenoids and their maximum absorbance peaks are localised at around 430 nm and 670 nm for chlorophyll a, around 450 and 640 nm for chlorophyll b and around 460 nm for carotenoids (Babichenko et al., 2001; De Muynck et al., 2009). Therefore, drops in reflectance should be observed at these specific wavelengths when biofouling is present.

Subsequently, lightness (L^*, black–white component), a^* (green–red component) and b^* (blue–yellow component) parameters as well as reflectance (%) for visible wavelengths (data every 10 nm) were obtained. From these data, ΔL^*, Δa^*, Δb^*, total colour difference (ΔE^*), chromatic variations (ΔC^*), changes in hue (ΔH^*) and fouling intensity (FI, %) were calculated using the following equations (De Muynck et al., 2009; Ferri et al., 2011; Tran et al., 2012):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$\Delta C^* = \sqrt{(a_x^*)^2 + (b_x^*)^2} - \sqrt{(a^*)^2 + (a^*)^2} \quad (2)$$

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2} \quad (3)$$

$$FI(\%) = (R_{700\ nm} - R_{670\ nm})_{x\ weeks,wet} - (R_{700\ nm} - R_{670\ nm})_{0\ weeks,wet} \quad (4)$$

where R_{700} and R_{670} are the reflectance of the sample at a wavelength of 700 nm and 670 nm and x is the time after the beginning of the test. Subsequently, photographs of the specimens were obtained with the use of a Canon Scan 3000F scanner for the image analysis and ImageJ 1.38x software was used to process them. De Muynck et al. (2009) proposed a quantification of the area covered by algae by means of a threshold analysis on the a^* and b^* coordinates of the CIELab colour space. Consequently, pixels with a* or b* values higher than the selected threshold were considered as unfouled, and the total amount of black and white pixels was then calculated by means of the *Analyze, Histogram* function. Visual comparison between photographs and both threshold analyses on the a* and b* coordinates, as well as histograms shows a better correlation using the threshold on a* coordinate. Consequently, pixels with a* values higher than 120 (0–255) were considered as unfouled spots. Subsequently, the percentage of area colonised by the algae could be calculated.

Finally, a non-destructive method for biomass quantification of algal biofilms was carried out. This method comprises the measurement of chlorophyll fluorescence by PAM-fluorometry (Eggert et al., 2006). First, a calibration of the method was carried out by means of correlating classic procedures (quantification of Chl a and dry weight) to minimum fluorescence of dark-adapted algae (F_0). Four replicates for each one of the 6 algal concentrations were tested to determine a standard curve of dry weight, measured by filtration on 0.45 μm filters. Two of the replicates were used to determine dry weight and the other two to measure F_0. In vivo Chl a fluorescence was determined with a pulse amplitude modulated fluorometer (PAM-2000, Heinz Walz GmbH, Germany) with a 6 mm aperture. After maintaining samples in dark conditions for 15 min, F_0 was determined placing the sample at a 90° angle and 7 mm from the filter. More specifically, 5 measurements in 30 s were taken with 600 Hz pulsed red measuring light (650 nm), measuring light intensity (ML) of 7 and gain (G) of 3. Afterwards, correlation between dry weight and F_0 was realized.

Samples placed in the accelerated algal fouling test were studied weekly for fluorometric parameters. Five measuring points were chosen on each mortar sample, one in the centre and one positioned at the border of each quadrant. First, 5 F_0 measurements in 30 s were taken and then, maximum quantum yield of Photosystem II, PSII (F_v/F_m), was determined for each point. This parameter gives information about the steady-state of quantum yield (Y) of PSII and consequently about the physiological status of the algae. Acceptable values of F_v/F_m are considered up to 0.5 according to Maxwell et al. (1994).

As a way to monitor the loss of viable algae due to hydric stress, completely fouled MPC samples (10 weeks of fouling) were maintained in the same conditions of light, RH (%) and temperature without provision of water/algal culture, with F_0 of the surface being recorded every week.

3. Results and analysis

3.1. Colorimetric measurements and image analysis

Fig. 1 shows a comparison of changes in reflectance among the six different degrees of bioreceptivity tested. As mentioned before, measurements were obtained weekly although not all the results are presented in Fig. 1 in order to provide a clear distinction between the curves. It is important to mention that the initial reflectance of MPC

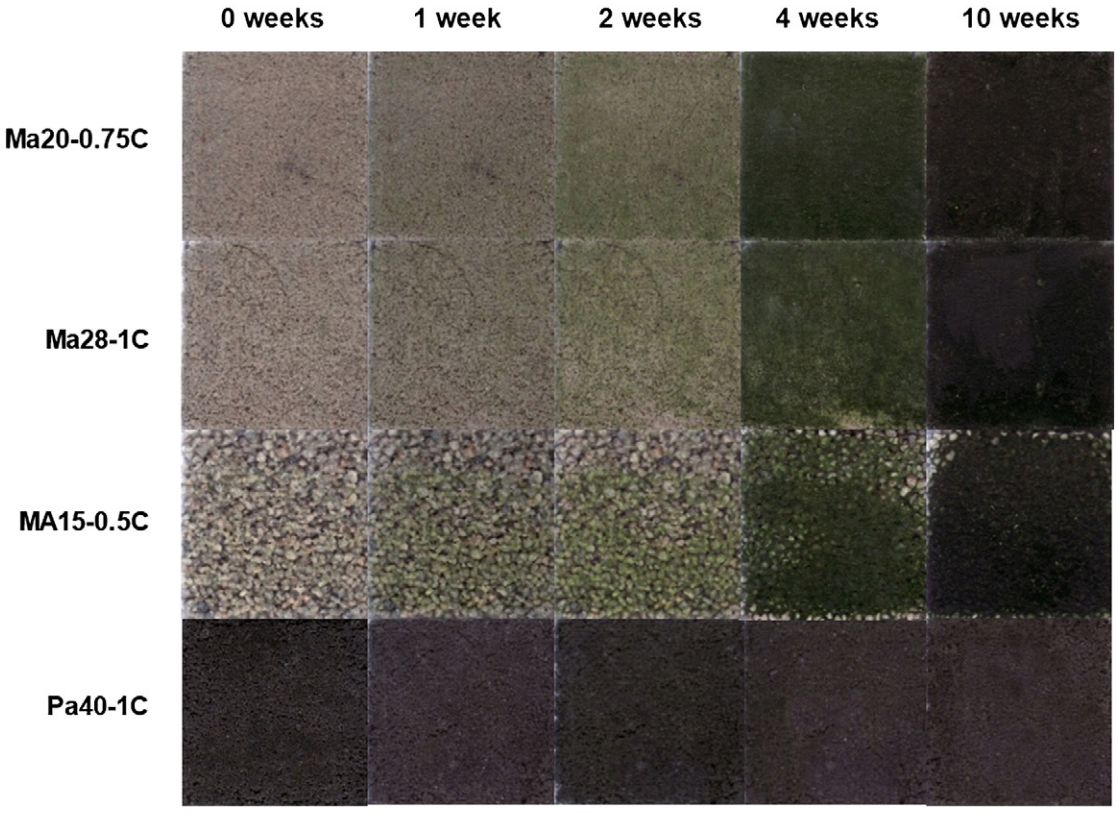

Fig. 2. Evolution of the visual appearance of MPC samples subjected to accelerated fouling and difference in initial colour between MPC and OPC specimens.

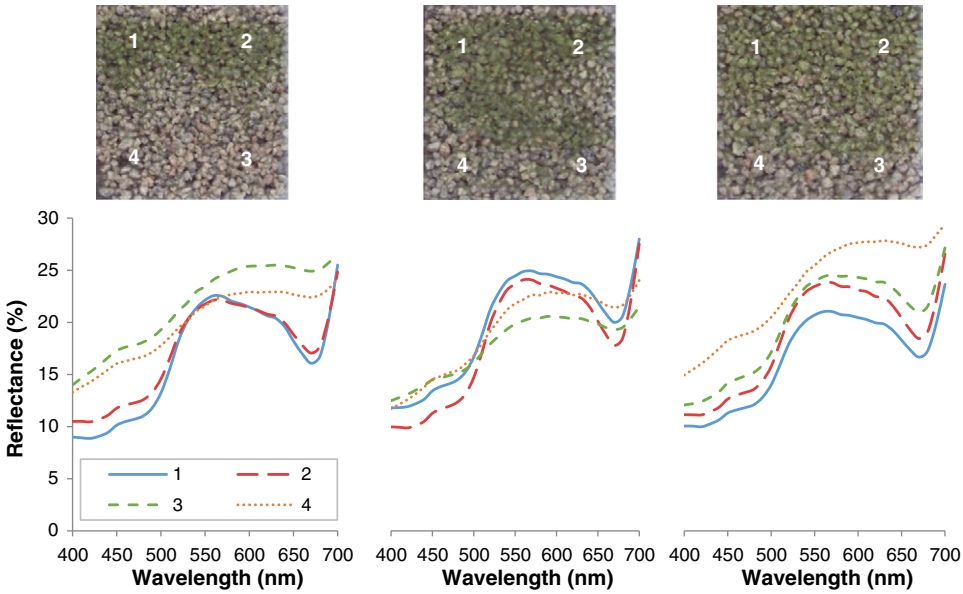

Fig. 3. Heterogeneity of reflectance curves for different points (indicated as 1, 2, 3, and 4) and replicates of MA15-0.5C specimens after 2 weeks of accelerated fouling.

samples was higher than for OPC ones due to the colour of the specimens' surface. As is evident from Fig. 2, MPC samples had an initial light brown colour while OPC samples were grey and darker. For this reason, the scales of reflectance for MPC and OPC graphs are different.

Completely dissimilar results were obtained for OPC and MPC mortars. Reflectance curves for the different MPC specimens were quite similar, recording the presence of *Chlorella vulgaris* after 1 week of exposure. Furthermore, the test was finished after 10 weeks of exposure for MPC samples. By this time, specimens were completely fouled and they were removed from the setup to study the response in dry periods. Visual inspections showed a completely fouled surface of some of the samples already after 4 weeks. From the fourth week until the end of the test accumulation of algae was observed, with a characteristic progressive darkening of the samples. Due to this change in lightness, an evident drop in reflectance was observed (Fig. 1).

From graphs of MPC samples (Fig. 1), it seems that there is no influence of porosity and roughness on biofouling. However, other tests as well as visual examinations showed a different reality. As mentioned before, four different points per specimen were analysed. Furthermore, considering that three replicates were studied, curves correspond to the average of 12 points. Consequently, initial heterogeneities are not detected as Fig. 3 illustrates. Independent of physical parameters, biofouling of MPC samples started in the upper part of the sample.

Results obtained for OPC samples were completely different. The test was prolonged until 20 weeks because no completely fouled surface was detected after 10 weeks. Reflectance curves from specimens prior to the test slightly differ among samples mainly due to the different amount of cement paste and w/c ratio. Biofouling was detected from the second week for Pa40-1C and PA30-1C, being the samples with the highest porosity and the highest roughness, respectively, although it was undetectable in a visual inspection. Furthermore, fewer reductions in reflectance were detected in comparison to MPC samples. This fact could be a consequence of the heterogeneity of the fouling of OPC samples as well as for the initial lower reflectance due to the natural colour of the samples. Pa60-1.75C samples showed no colonisation on their surface although the presence of algae was detected by means of colorimetric measurements due to the accumulation of the sprinkling water in some small cavities (as a consequence of air voids). Concerning PA30-1C samples, it was detected that algae progressively colonised spaces between aggregates due to the high surface roughness. Furthermore, it is important to notice that biofouling of OPC samples started at the bottom of samples' surfaces. This fact may be mainly a consequence of different humidity conditions between the upper and the lower part of OPC specimens.

Other parameters obtained and estimated from colorimetric measurements are shown in Table 2. Parameters of interest related to the current objective are parameters L^* and a^*, and total difference in colour. Significant changes in lightness were observed for OPC samples, although bigger differences were obtained for MPC samples. Similarly, parameter a^* showed a progressive drop, indicating more or less intense biofouling except for Pa60-1.75C. This fact corresponds with previously analysed results, showing no biofouling on these samples.

Table 2
Colorimetric measurements.

	Cycle	L^*	a^*	b^*	ΔE	ΔC	ΔH
Pa40-1C	0	37.45 ± 1.10	−0.02 ± 0.12	4.21 ± 0.40			
	20	29.59 ± 1.56	−3.22 ± 0.59	7.49 ± 1.04	9.1	3.94	2.34
Pa60-1.75C	0	43.91 ± 1.57	−0.43 ± 0.10	3.71 ± 0.48			
	20	38.34 ± 1.57	1.34 ± 0.67	10.13 ± 1.78	8.69	6.49	1.51
PA30-1C	0	37.26 ± 2.31	−0.49 ± 0.07	2.34 ± 0.37			
	20	26.79 ± 2.51	−3.18 ± 0.54	7.63 ± 1.23	12.04	5.88	0.84
Ma20-0.75C	0	55.88 ± 0.95	2.29 ± 0.18	10.93 ± 0.49			
	10	23.02 ± 1.63	−3.12 ± 0.71	9.83 ± 1.50	33.32	−0.85	5.46
Ma28-1C	0	57.08 ± 1.30	2.48 ± 0.24	10.81 ± 0.43			
	10	19.29 ± 1.06	−4.50 ± 0.36	7.55 ± 1.05	38.57	−2.30	7.35
MA15-0.5C	0	57.29 ± 2.22	1.42 ± 0.53	9.24 ± 1.00			
	10	23.55 ± 5.46	−4.30 ± 0.85	12.59 ± 3.45	34.38	3.95	5.32

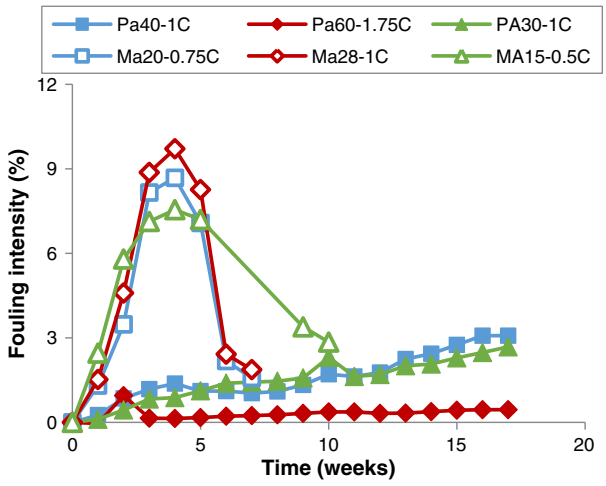

Fig. 4. Evolution of fouling intensity (FI). Range of standard errors per dosage during the test: Pa40-1C: 0.19–1.67%; Pa60-1.75C: 0.01–0.26%; PA30-1C: 0.01–0.38%; Ma20-0.75C: 0.06–0.25%; Ma28-1C: 0.14–0.35%; and MA15-0.5C: 0.15–0.47.

Furthermore, higher total differences in colour where detected for MPC samples, Ma28-1C being the one showing the highest values. This fact might indicate that Ma28-1C specimens are the most bioreceptive ones of all studied samples.

Subsequently, fouling intensity, FI (%), was estimated as mentioned in Subsection 2.3. Fig. 4 does not show results corresponding to weeks 6, 7 and 8 due to a problem during the tests, which led to meaningless results. Nevertheless, the graph clearly shows the trend of biofouling. First, MPC samples exhibited higher values and a faster increase of the fouling intensity parameter compared to OPC samples (Fig. 4). Initially, a fast rise of fouling intensity was observed for all MPC samples. Highest values were obtained for Ma28-1C, arriving to around 10% and concurring with the reflectance spectra mentioned before. However, a notable drop was observed after week 4. This indicates that FI (%) is a useful parameter during the covering process, but can lead to misinterpretation when surfaces are already completely fouled. In the particular case of MPC samples, they were completely fouled after 4 weeks of testing. After these cycles, accumulation of algae on specimens' surface was observed, with the result that surface colour was becoming darker and reflectance was decreasing. In the FI formula, differences between reflectance at 670 nm and 700 nm are considered. Therefore, decreasing the overall reflectance of the sample implies a reduction of this difference (as can be seen in Fig. 1) and FI ceases to represent the real evolution of biofouling (Fig. 4).

Additionally, OPC samples showed a constant increase trend in fouling intensity although slower than for MPC specimens. Furthermore, this trend may continue further since no complete fouling was observed until 20 weeks. However, the fouling intensity at 20 weeks obtained for OPC samples was reached already after 2 weeks for MPC samples.

Fig. 5 illustrates the fact that MPC samples suffered an important drop in reflectance curves in comparison with OPC specimens. This progressively darker colour as a consequence of algae accumulation for all MPC specimens indicates that both pH and chemical composition are more suitable for allowing colonisation of *Chlorella vulgaris*. Decrement in lightness (ΔL^*) is proposed as a parameter to show a major or minor degree of algal accumulation after complete fouling of specimens' surface.

Subsequently, Fig. 6 shows the changes in fouled area. Again, the higher bioreceptivity of MPC samples is evident. As mentioned before, a quick fouling of MPC samples was recorded until complete fouling. Furthermore, the area of fouling for MPC samples increased until around 90%, while for OPC specimens it remained always lower than 50%. Nonetheless, it is important to remark that it is not possible to obtain a 100% of fouling due to pixels corresponding to open porosity. After the sixth week of testing, some incongruent results were recorded due to brightness of the photographs. Moreover, the higher the amount of algae on the surface, the larger the retention of water. Consequently, humidity conditions as well as changes in lightness of the samples caused difficulties to record the evolution of the area of fouling without changing the threshold parameter and/or coordinate.

3.2. Biomass quantification

The calibration curve for the non-destructive biomass quantification method is shown in Fig. 7. Correlation between dry weight and F_0 values follows an exponential tendency and this is coherent with CO_2 availability for the cells in dark conditions.

The first stage of the curve corresponds to the fact that cells form a monolayer. In this stage, a linear tendency is observed until there is no more space available and cells are starting to form a multilayer conformation. In this stage, depending on the thickness, just a portion of cells would be in contact with environmental CO_2, and consequently, this method would not be suitable for biomass quantification. Consequently, limit of suitability of the current method should be considered at around 320 mg/L (20 mg/cm^2).

Fig. 8 also shows the correlation between F_0 and biomass expressed by area (cm^2). This correlation should be used with caution because no

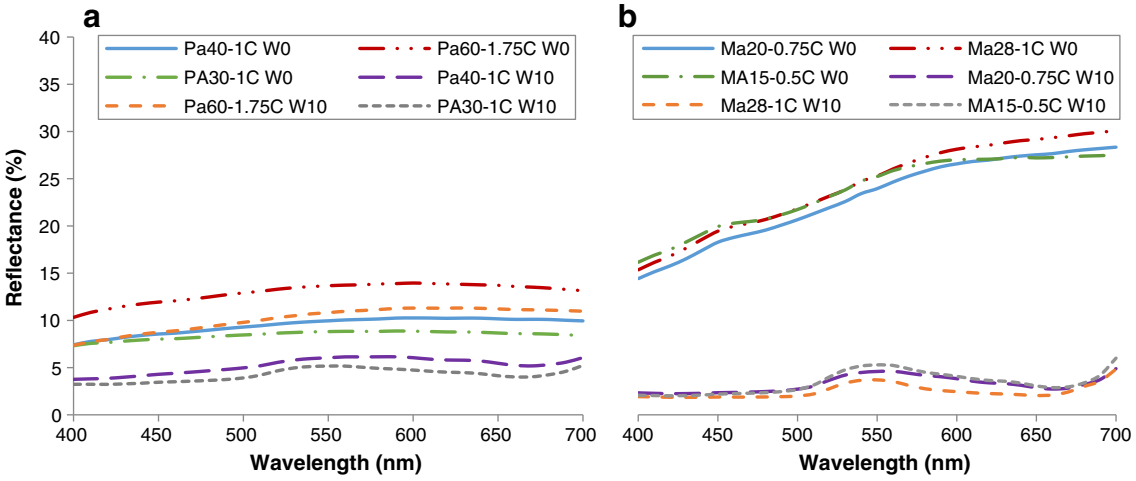

Fig. 5. Differences in reflectance curves of OPC (a) and MPC samples (b) prior to and after 10 weeks of accelerated fouling.

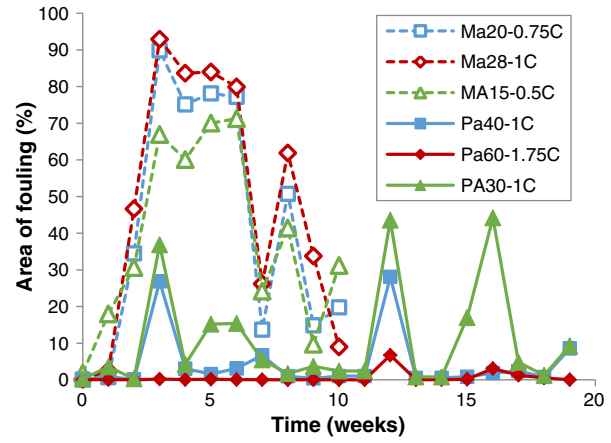

Fig. 6. Evolution of the area of biofouling. Range of standard errors per dosage during the test: Pa40-1C: 0.01–11.16%; Pa60-1.75C: 0.01–1.02%; PA30-1C: 0.03–5.16%; Ma20-0.75C: 0.04–7.84%; Ma28-1C: 0.05–13.67%; and MA15-0.5C: 0.33–10.56%.

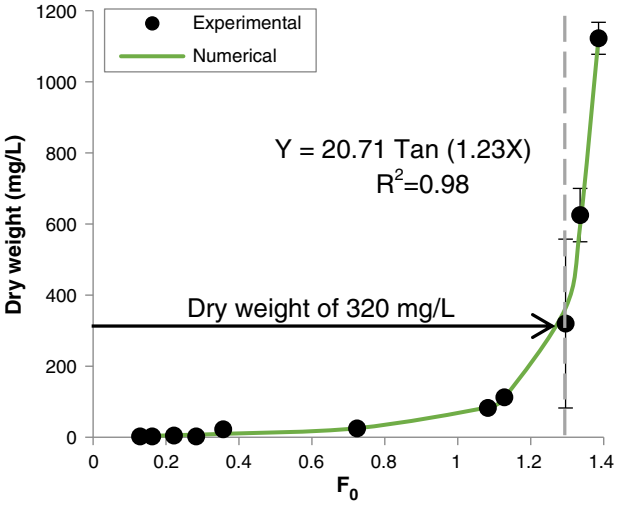

Fig. 7. Standard curve for biomass quantification by means of a correlation between dry weight and F_0.

homogeneous growth is observed in cementitious materials. However, a quantification of dry weight expressed by mg/L has no sense when the surface of building materials is studied. Furthermore, due to heterogeneity of the material and size of *Chlorella vulgaris*, it was decided not to use the basic international units system (using mg/cm^2 instead of mg/m^2).

Fig. 8 shows biomass quantification taking into account the correlation between dry weight and F_0. The most important and evident results are that Ma28-1C specimens showed the maximum amount of biomass on their surfaces, and no biofouling was recorded on Pa60-1.75C specimens.

In comparison with the OPC samples, MPC samples showed a faster and more marked increase of biomass. Additionally, the lower biomass observed on MA15-0.5C might be the consequence of higher pore diameter. It was visually detected that sprinkling water (with nutrients and algae) penetrated through the pores and less retention of algae was recorded. Furthermore, between samples with the same size of aggregates, a higher response to biofouling was observed for Ma28-1C. This fact was also shown in other tests such as fouling intensity, area of fouling (until total coverage of specimens' surface) and ΔE.

Table 3 presents an average of F_v/F_m values obtained from weeks 1 to 5. The algae covering both Pa40-C and PA30-C samples exhibited good physiological state in terms of photosynthesis from the third and fourth weeks onwards respectively (F_v/F_m values higher than 0.5 as mentioned before). The above could indicate that Pa40-1C specimens presented a slightly higher bioreceptivity for *Chlorella vulgaris* than PA30-1C. Additionally, no algal presence was recorded in Pa60-F specimens by means of colorimetric measurements although a low level of dry weight was recorded. F_v/F_m values also indicate the presence of an extremely low amount of algae. These results showed that the algae were under stress (the substrate is not the appropriate) and indicated the low accuracy of the method. MPC specimens presented a higher response, especially for Ma20-0.75C and Ma28-1C specimens, while the values for MA15-0.5C specimens were somewhat lower.

Finally, the influence of a dry period on biomass evolution was evaluated. Fig. 9 shows F_0 values obtained from 1 to 9 weeks in dry conditions. This graph clearly presents three different stages. First, a drop of F_0 values was observed, which may be a consequence of a decrease in biomass or a protective mechanism to overcome the lack of water, with the algae becoming less photosynthetically active. According to Heber et al. (2007), F_0 for dark-adapted mosses or lichens decreases when the drying process occurs slowly. This reduction of F_0 took place during the first week due to water stress. Subsequently, the response to fluorescence increased until the second week, which can be considered as a stress indicator similar to what is observed for the case of plants (Lichtenthaler, 1988; Rohácek, 2002). The final decrease of F_0 after week 2 corresponded in this case to the death of the algae (drop of biomass). Furthermore, F_v/F_m values revealed that this method should not be used as a quantification technique when data lower than 0.2 are obtained (Tables 3 and 4).

Taking into account the results obtained for F_0 and F_v/F_m, it was observed that until one week of water stress, a protective mechanism

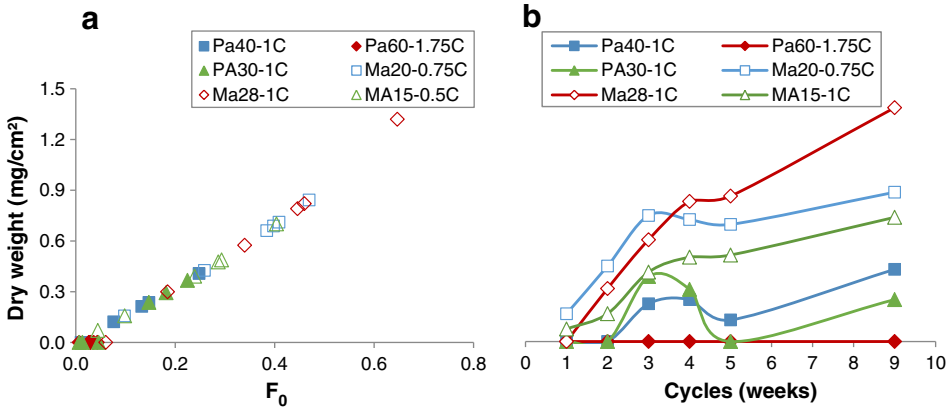

Fig. 8. MPC samples exhibited a higher amount (a) and a faster rate (b) of biomass accumulation during the fouling tests compared to OPC samples.

Table 3
F_v/F_m values obtained between cycles 1 and 5 indicating the state of the photosynthetic apparatus.

Cycle (weeks)	Pa40-1C	Pa60-1.75C	PA30-1C	Ma20-0.75C	Ma28-1C	MA15-0.5C
1	0.289 ± 0.063	0.198 ± 0.050	0.380 ± 0.055	0.494 ± 0.008	0.438 ± 0.025	0.334 ± 0.017
2	0.434 ± 0.041	0.152 ± 0.037	0.427 ± 0.048	0.578 ± 0.003	0.502 ± 0.022	0.487 ± 0.011
3	0.532 ± 0.034	0.171 ± 0.031	0.489 ± 0.052	0.617 ± 0.006	0.581 ± 0.013	0.577 ± 0.007
4	0.622 ± 0.006	0.139 ± 0.029	0.616 ± 0.005	0.553 ± 0.011	0.597 ± 0.005	0.511 ± 0.014
5	0.533 ± 0.022	0.179 ± 0.038	0.526 ± 0.013	0.530 ± 0.010	0.534 ± 0.009	0.485 ± 0.010

could be occurring, leading to a decrease of both parameters. However, during the second week of water stress, F_0 underwent a sharp increase while F_v/F_m continued decreasing. According to some authors (Heber et al., 2001; Souza et al., 2004; Guo et al., 2006), the above may be related to an advanced stage of stress where PSII is damaged. Finally, in this particular case where no water was applied for 2 weeks, irreversible damage was observed provoking the death of the algae.

4. Conclusions

MPC specimens showed a higher bioreceptivity for *Chlorella vulgaris* than OPC specimens. This may be a consequence of the pH and chemical composition which makes this hydraulic binder suitable to allow growth of micro-organisms. However, influence of roughness and porosity should not be discarded although they seem to have less influence on the basis of the results obtained. Furthermore, Ma28-1C appeared to be the most bioreceptive MPC composition, in spite of having the lowest porosity of all samples, and a lower surface roughness than MA15-A.

MPC is more suitable for stimulation of algal colonisation. However, other groups of organisms may have higher affinity for carbonated OPC. Nevertheless, since algae are pioneers of biofouling of cementitious materials, together with bacteria, MPC mortar is likely to be more rapidly colonised. Furthermore, it was observed that Ma28-1C specimens were the most suitable ones in order to stimulate colonisation of *Chlorella vulgaris* in laboratory conditions. Chemical properties of these specimens seem to have more influence on colonisation than physical properties did. This fact can be seen when comparing OPC and MPC samples. Furthermore, the clearest difference between different MPC specimens for *Chlorella vulgaris* colonisation is the heterogeneity of initial growth. Due to the higher pore diameter, water containing the algae has entered into the specimens, showing a more heterogeneous fouling pattern. MPC specimens with lower roughness showed similar results although visual inspections showed a more homogeneous and resistant growth and better aesthetic appearance during water stress for MPC specimens with the lowest porosity.

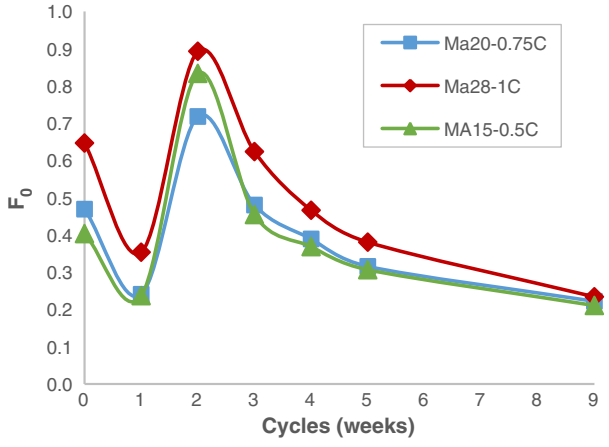

Fig. 9. F_0 response to hydric stress. Range of standard errors per dosage during the test: Ma20-0.75C: 0.003–0.008%; Ma28-1C: 0.004–0.014% and MA15-0.5C: 0.006–0.026%.

Visual inspections revealed a difference in the first stage of colonisation between OPC and MPC specimens. For OPC samples, biofouling started at the bottom side of the specimens, which may be related to the high amount of moisture located in this area. In contrast, MPC specimens showed a better water retention as well as more homogeneous moisture distribution. Consequently, the MPC mortar samples behave like a filter where water penetrated and algae remained on the surface, initially localising algal fouling in the upper part.

Related to the initiation of biofouling, the presence of algae was detected from the first week on MPC specimens' surface and from the second week on OPC ones. Moreover, MPC samples were completely fouled after 4 weeks, while no complete fouling was observed until 20 weeks for OPC samples.

Concerning the techniques and evaluation criteria used, some considerations should be taken into account. First, results obtained from the fouling intensity analysis revealed that this parameter is not suitable when MPC samples are completely fouled. Once the specimen's surface was covered, the amount of algae was increasing forming different layers and showing as a result a decrease in lightness (L^*). The highest decrement in lightness was observed for MPC specimens, resulting in a drop of "fouling intensity" in the range between 62% and 82%. Additionally, a more pronounced decrease of the a^* parameter was also observed for MPC samples. A threshold on the a^* parameter was used for image analysis and pixels with a^* values higher than 120 were considered as fouled. However, problems to obtain realistic values were observed after the sixth week of testing. Once MPC specimens were completely fouled and the thickness of the algae layer was increasing, lightness was decreasing and humidity of the samples was increasing. Consequently, humidity caused some brightness in the photographs that made them more difficult to analyse. The current method for algal quantification is suitable until biofouling reached a coverage of about 20 mg/cm^2. Higher amounts of dry weight induce low and insignificant differences on F_0. The mg/cm^2 units are proposed in order to express biomass for surfaces, since expressing biomass relative to volume units is not appropriate for this purpose.

Limitation of the use of this method was also observed when algae were submitted to dry periods. Increases of F_0 values were observed where exposed in dry periods probably showing PSII damage concurring with a drop in F_v/F_m values.

This study indicates the feasibility of magnesium phosphate cement as a binder for the development of green concrete structures.

Acknowledgements

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Table 4
F_v/F_m values obtained between cycles 1 and 5 under hydric stress.

Cycle (weeks)	Ma20-0.75C	Ma28-1C	MA15-0.5C
1	0.133 ± 0.013	0.031 ± 0.005	0.055 ± 0.009
2	0.012 ± 0.005	−0.019 ± 0.002	−0.011 ± 0.005
3	−0.020 ± 0.004	−0.027 ± 0.001	−0.016 ± 0.003
4	−0.026 ± 0.002	−0.030 ± 0.002	−0.023 ± 0.002
5	−0.018 ± 0.002	−0.025 ± 0.003	−0.020 ± 0.004

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