Construction and Building Materials 199 (2019) 396–405

Contents lists available at ScienceDirect

Construction and Building Materials

journal homepage: www.elsevier.com/locate/conbuildmat

Effect of temperature and relative humidity on algae biofouling on different fired brick surfaces

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HIGHLIGHTS

- Microalgae biofouling on different porous and rough fired brick surfaces was studied.
- Its growth under different values of relative humidity and temperature was tested.
- At relative humidity lower than 98% no growth was present.
- Temperature influenced algae growth rate and covered area as a function of time.
- Biofouling experimental results were modelled by a modified Avrami's law.

ARTICLE INFO

Article history:
Received 3 August 2018
Received in revised form 4 December 2018
Accepted 5 December 2018

Keywords:
Algal growth
Building material biodeterioration
Temperature
Relative humidity
Porosity
Roughness

ABSTRACT

The purpose of this study was to evaluate the effect of environmental temperature and relative humidity on algae biofouling that often occurs on porous and rough fired brick surfaces. Brick samples were chosen since their common use on building façades. Accelerated growth tests were performed under different relative humidities and different temperatures. Results showed the effects of different temperature conditions in terms of algae growth delay and reduction of the covered area. All the relative humidity conditions tested substantially showed no growth from an engineering standpoint. The modified Avrami's law succeeded in modelling the biofouling under the different environmental conditions.

1. Introduction

Biodeterioration can be defined as "an undesirable change in the properties of the material caused by living organisms" [1]. It is a very common phenomenon on façades, especially on fired brick surfaces, since it is a material commonly used as cladding on external walls [2–5]. A complex community of microorganisms such as algae, lichens, moulds, fungi and bacteria can form a biofilm on material surfaces when exposed to natural weathering [6–8]. The biological matrix in contact with a building material can modify its chemical, physical and mechanical properties, as well as the mechanisms responsible for deterioration [9,10]. Among these organisms, green algae and cyanobacteria are the first colonisers since they are present in the environment as spores, cells and fragmented filaments [11,12]. Moreover, as they are autotrophic, they only need sunlight for their growth, and, due to their morphological and physiological adaptations, they can adapt to extreme environments, even surviving to desiccation processes [13,14]. Microalgae represents a group of very versatile microorganisms. It is therefore known that the growth rate of green algae and cyanobacteria is largely dependent on the species, their degree of competitiveness in natural environments and some other environmental factors (e.g., aeration, irradiance, temperature, growth medium, etc.,) [14–16]. Recent studies point out that the bioreceptivity of a building material is mainly affected by two parameters: water availability, related to environmental conditions (relative humidity, temperature, wind driven rain, rising damp) [17,18]

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https://doi.org/10.1016/j.conbuildmat.2018.12.023
0950-0618/

and both physical and chemical substrate properties, such as surface roughness, porosity, pH and mineralogical composition [19,20]. For phototropic microorganisms, such as green algae and cyanobacteria, both physical and environmental factors involved in the colonisation of building façades have been identified, but only few studies highlight the effects of these factors on the microbial growth [11,21]. Up to now, researches have only revealed the influence of the physical parameters of the substrate: porosity helps the retention of water and nutrient for algal growth, while roughness favours the mechanical grip of algae to the substrate itself [22–24].

In literature, several studies propose numerical modelling of the microbial growth in order to predict the biofouling on building components. Failure models about mould growth are highly investigated [17,25,26], while those about growth of microalgae are scarce and mainly related to the Avrami's model at fixed optimal temperatures [22,27,28]. Thus, further researches are needed to investigate the effect of different environmental conditions, such as non-optimal temperatures and relative humidities. In this way, this paper aims to analyse, through accelerated tests on fired brick samples, with different total porosity and roughness, the influence of different environmental temperatures and relative humidities on growth of microalgae and cyanobacteria. Subsequently, the predicting capability of a modified Avrami's model has been verified on the experimental results.

2. Materials and methods

2.1. Phases

This work is divided in three phases. A preliminary step for choosing temperature to be used during the tests was firstly performed. Then, experimental tests were performed under one, previously selected, constant temperature to understand the influence of different relative humidities on growth of microalgae and cyanobacteria, as well as, under different temperatures at a constant relative humidity to understand the role of temperature. The role of the substrate was also investigated. The last phase involved the modelling of the experimental results.

2.2. Materials

Three types of clay bricks (named A, B and C) were selected. Biofouling on the tested materials was investigated both on original rough surfaces (R) and on manually smoothed surfaces (S) by sand paper. To evaluate the effect of the substrate, clay bricks were preliminarily characterized. Total porosity P [%] of each material was determined from 3 samples by a mercury intrusion porosimeter (Micromeritics Autopore III) according to the ASTM D4404-10 standard [29]. The surface roughness R_a [μm] was measured according to UNI EN ISO 4287:2009 standard [30], by using a Taylor Hobson CCI 3D Optical Profiler. The arithmetical mean deviation of the assessed profile was calculated on five sampling lengths of 5,54 mm.

A green alga (*Chlorella mirabilis* strain ALCP 221B) and a cyanobacterium (*Chroococcidiopsis fissurarum* strain IPPAS B445) were chosen for the experimental test, since they can be commonly found on building façades in European countries [31–33]. Microbial strains were cultivated as pure cultures in 5-L glass flasks containing Bold's Basal medium (BBM), prepared in accordance with ASTM D5589-09 standard method [34]. Cultures were both incubated at 24 °C, under a light intensity of 1500 lux with a 14/10 h light/dark photoperiod. The mixed cultures to be used in the experimental assays were obtained by mixing the two pure cultures in a ratio 1:1 (v/v).

2.3. Methods

2.3.1. Preliminary test about the influence of temperature on algae growth

The effect of temperature on the growth of the tested strains with no brick substrate had previously been tested in order to set the environmental temperature during the further accelerated tests. According to the available literature, algae are capable of growing in a wide range of temperature usually comprised between 5 and 40 °C [35,36]. This way, both pure and mixed cultures were incubated at the following temperatures: 5 ± 2.5 °C, 10 ± 2.5 °C, 27.5 ± 2.5 °C, 35 ± 2.5 °C and 40 ± 2.5 °C. The results were comparatively evaluated. Growth tests were carried out using glass bottles containing 100 mL of each culture to be tested and incubated in a refrigerated thermostat (Velp FOC 215E). Since microalgae and cyanobacteria need light to grow, a controlled daylight intensity of 1500 lx was supplied for day/night cycles equivalent to 14/10 h. The growth chamber was equipped with a 39 W neon lamp (Sylvania TopLife) characterized by a light temperature of 5000 K [9]. Temperature and relative humidity in the test environment were continuously measured with sensors (Sensirion SHT31-D) placed inside the incubators. Cultures were sampled every week and subject to microscopy counting using a Thoma-Zeiss hemocytometer [37]. The results were expressed in logarithmic scale as number of cells/mL. All the tests were performed in duplicate.

2.3.2. Accelerated growth tests under different relative humidities

The study of biofouling on building materials may raise some methodological problems concerning its occurrence as readily observable and quantifiable phenomenon [38], since a visible biological degradation mostly starts after 1-year or more of natural exposure [39,40]. Hence, the use of accelerated tests is generally recommended.

Three different relative humidity (RH) conditions were reproduced in three separate climatic chambers to investigate their effect on growth of microalgae and cyanobacteria on fired brick surfaces. The indoor environment was conditioned by saturated solutions, as indicated in EN ISO 12571:2013 [41]. Incubators consisted of three 100 × 40 × 53 cm^3 glass chambers, each one filled with 15 L of saturated solution (Fig. 1a). The RH_1 (about 75%) was obtained through a saturated solution of NaCl, RH_2 (about 87%) through a saturated solution of Na_2CO_3, and RH_3 (about 98%) through only deionized water [42]. To consider exclusively the effect of RH, temperature was maintained at 27.5 ± 2.5 °C during all the tests. This temperature was selected based on the results of the preliminary test (see Section 2.3.1), from which an optimal temperature for growth was estimated according to literature [43–45] too. Environmental conditions inside each glass chamber were monitored. Measurements were recorded every 10 min over the entire period. Three prismatic samples (8 × 8 × 3 cm^3) for each tested material were investigated in each environment until the end of the process (stagnation phase) was reached. At the beginning of the test, 9 different points on the surface of each sample were inoculated with 5 μL of the mixed culture per point. After the initial inoculation, samples were positioned inside the climatic chambers, inclined at 45° on aluminium-glass racks, front-to-front along the long dimension of the chamber. The test apparatus was placed in a closed room to avoid the influence of light, temperature and RH of the external environment. Each growth chamber was equipped with two neon lamps (Sylvania TopLife 39W) to provide an adequate illumination equivalent to day/night cycles 14/10 h. The lamps were positioned at a constant distance from the sample surface.

2.3.3. Accelerated growth tests under different temperatures

Investigations on the influence of temperature on algae growth was carried out following previous researches [22,33,46,47]. Accelerated tests with a periodical water spray on the material surface were performed until the stagnation phase

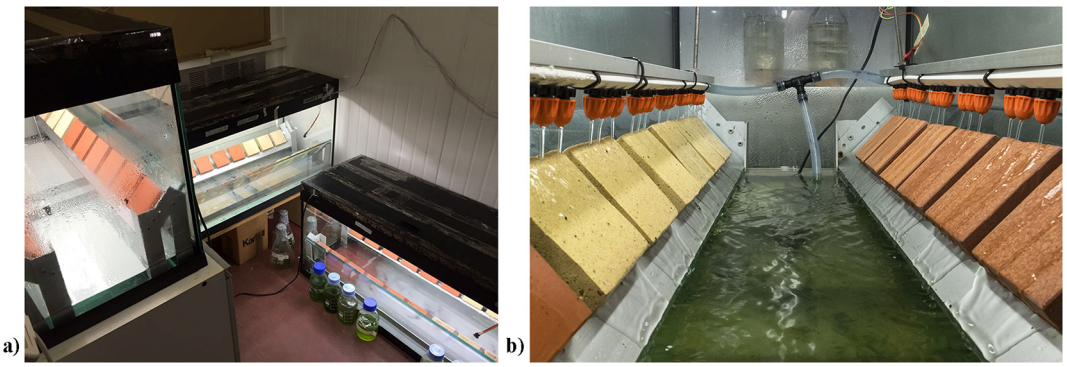

Fig. 1. a) Test apparatus for the evaluation of relative humidity influence on growth process; b) Test apparatus for accelerated test aimed at temperature effect investigation.

was reached (Fig. 1b). Test apparatus consisted of growth chambers ($100 \times 40 \times 53$ cm^3), filled with 35 L of BBM inoculated with the mixed cultures. Algal suspension was sprinkled on sample surfaces (8×8 cm^2) positioned above two aluminium-glass composed racks inclined at 45°. Run/off cycles were set for a duration of 15 min and a total of 6 h per day (3 h run and 3 h off). A day/night illumination cycles (14/10 h) were provided by two 39 W neon lamps (Sylvania TopLife).

From the results of the preliminary growth tests under different temperatures and from the available literature [35,36,43–45,47–49], the accelerated tests were set under two different temperatures: 27.5 ± 2.5 °C, that is a temperature within the range of the optimal growth values comprised between 20 °C and 30 °C [43–45,47–49], and a lower value equal to 10 ± 2.5 °C, within the range of suitable growth for both the strains under study [35,36]. To set the lower test temperature a modified refrigerator (Electrolux RC 5200 AOW2) was used. Relative humidity was assumed constantly equal to 100% due to the wetting cycles. All the test environments were monitored by temperature and RH sensors (Sensirion SHT31-D), through measurements every 10 min.

2.3.4. Measurements and evaluation of algae growth

During each accelerated growth test, both qualitative and quantitative analyses were carried out for the evaluation of the algal extent and the biofouling process on samples' surface [46]. Firstly, a (qualitative) colorimetric analysis was performed to examine the colour variation during time. Colorimetric measurements for the evaluation of the chromatic variation (ΔE) were carried out with a spectrophotometer (Konika Minolta CM-2600dD) [33,50]. In accordance with UNI EN 15886:2010 and UNI 1602371:2018, results were expressed in CIELAB colour space [51,52]. Colour variation was calculated in terms of total colour difference ΔE, by Eq. (1):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

where L_0^*, a_0^* and b_0^* are the colour coordinates of samples before the beginning of the test (time zero), and L^*, a^*, b^* the ones measured during the accelerated growth. However, according to standard methods [52] and researches [53], only a total colour difference $\Delta E < 1$ is not detectable for naked human eyes, while a ΔE between 1 and 2 is visible only after a close observation. From an engineering standpoint, a $\Delta E = 1$ could be assumed as the acceptable lowest bound for algae growing. In case of average $\Delta E > 1$, the calculation of a_0^*–a^* was also determined to verify if the colour variation was due to the presence of algae. Δa^* corresponds to the red/green difference, and it permits to associate the colour variation to algae appearance in terms of green stains: the amount of red is indicated by positive values ($\Delta a^* > 0$), while a green toning by negative values ($\Delta a^* < 0$). In this way, if on average $1 < \Delta E < 2$ but on average $\Delta a^* > 0$, it can be reasonably assumed that the colour variation is not due to algae growth. Measurements were repeated on nine points on each sample surface about every week.

The quantification of the biofouling extension was evaluated by (quantitative) digital image analysis (DIA). The effectiveness of this method has been confirmed in previous studies [12,46]. A high-resolution scanner (HP Scanjet G3010) was used for the acquisition of suitable images. Scanned images of the samples with a resolution of 600 dpi were used to calculate the algal coverage, expressed as a percentage of the total sample area. The acquired images were elaborated with ImageJ software [54,55], and, after a binary conversion, pixels representing the contaminated area by microalgae were counted. The covered area was expressed as a percentage (0% corresponded to null-growth, while 100% to the completed coverage of the sample area). The quantitative measurements of the colonized area were weekly carried out during the accelerated growth tests and results were reported as average values and standard deviations of three samples for each tested material.

Scanning Electron Microscope (SEM) observation was carried out onto representative samples to have an insight into the interactions among algae and cyanobacteria, and the tested fired brick surfaces collected after the accelerated growth tests. In order to preserve the biological material before SEM observation, all the brick surfaces were subject to the treatments proposed by Gao et al. [56] except for the post-fixation step with osmium tetroxide [57].

Briefly, a portion of selected bricks was fixed in 30 g/L glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 4 h at 25 °C. Samples were subsequently submerged in phosphate buffer for 15 min three times. Samples were dehydrated in ethanol at the following concentrations: 15%, 30%, 50%, and 70% for 10 min each, 85% and 95% for 15 min each, and 99.5% for 1 h. Samples were gold coated in a sputter coater K550X (Emitech, Ashford, United Kingdom). Finally, the brick portions were observed using a XL30 SEM (Philips Amsterdam, Netherlands) equipped with a ES-423 extended life lanthanum hexaboride LaB6 cathode. The investigated clay bricks were the type AR and CR, collected after accelerated growth tests at 27.5 ± 2.5 °C (optimal growth condition), in order to take into account the lowest and highest porosity and different roughness.

2.3.5. The modified Avrami's model

As already used in previous studies under optimal growing conditions [28,47,58], the modified Avrami's model, shown in Eq. (2), was adopted to express the algae growth under non-optimal conditions, too. This allows to consider a not full coverage of the sample area.

$$X(t) = (1 - \exp^{-K(t-t_1)^n}) \cdot \frac{A_c}{A_t} \quad (2)$$

The covered area $X(t)$ [%] is a function of time t [day], K is a growth rate factor [–] depending on the nucleation rate of algal cells, t_1 is the latency time [day] and it corresponds to the time until $X(t) = 0.3\%$, and n is a coefficient that can be assumed equal to 4 [28]. The final covered area ratio A_c/A_t [%] is calculated from the covered area A_c by algae at the end of the test and the total area A_t of the sample. The parameters A_c/A_t and t_1 were determined for each material as the average values between measurements from 3 samples. K was calculated through iterations by minimizing the least squares value between experimental data and calculated values. In order to verify the accordance between experimental measurements (tests under 10 °C and 27.5 °C) and analytical values, a confidential R factor, was calculated as in Eq. (3).

$$R = \sqrt{\frac{\sum_{t=1}^{m} (X_{an} - X_{ex})^2}{\sum_{t=1}^{m} X_{ex}^2}} \cdot 100 \quad (3)$$

where X_{an} and X_{ex} represent the analytical and the experimental colonization area at time t, respectively [27]. A low R value indicates a good agreement between the calculated biofouling and the real colonization [47].

3. Results and discussion

3.1. Characterization of substrate properties

The substrate properties of each tested samples are summarized in Table 1. Among the tested fired bricks, samples A were found to be the lowest porous material, whereas samples C showed the highest porosity. Sample B exhibited the lowest roughness, while on samples A and C the smoothing operation reduced the roughness of about 1 μm. In this way, the effect of different substrates was investigated.

3.2. Results of preliminary algae growth tests under different temperatures

Growth curves of the pure (CM or CF) and mixed (MIX) cultures at the different temperatures are reported in Fig. 2. Regarding *Chlorella mirabilis* (CM) (Fig. 2a), an increasing number of cells was observed at both 10 and 27.5 °C. Hence a growth process was observed in accordance with observations reported by Shukla et al. [15] that studied the growth of *Chlorella mirabilis* in low-temperature environments. The number of cells at T = 5 °C was constant over the time, with loads attesting at about10^5 cells mL^{-1}. The higher tested temperatures (35 °C and 40 °C) lead to a remarkable decrease in the cell numbers of the tested strains, with a reduction of cells starting after 14 days of growth monitoring. Regarding the growth of *Chroococcidiopsis fissurarum* (CF) (Fig. 2b), no particular effect of the tested temperatures was observed at the end of the monitoring, thus confirming the high adaptation of this blue algal species to extreme environments [16]. Finally, the growth curves recorded for the mixed cultures (Fig. 2c) substantially reflected the trends of the growth curves related to the two pure cultures. It is noteworthy that the cell counting method used in this study did not allow to establish the prevalence of one species over another. It is therefore known that,

Table 1
Properties of tested clay brick samples (mean value ± standard deviation).

Sample	Total porosity [%]	Roughness [μm]
AR	19.24 ± 0.37	5.54 ± 0.42
AS	19.24 ± 0.37	4.50 ± 0.27
B	24.62 ± 1.02	2.95 ± 0.63
CR	44.09 ± 1.63	7.60 ± 0.57
CS	44.09 ± 1.63	6.60 ± 0.49

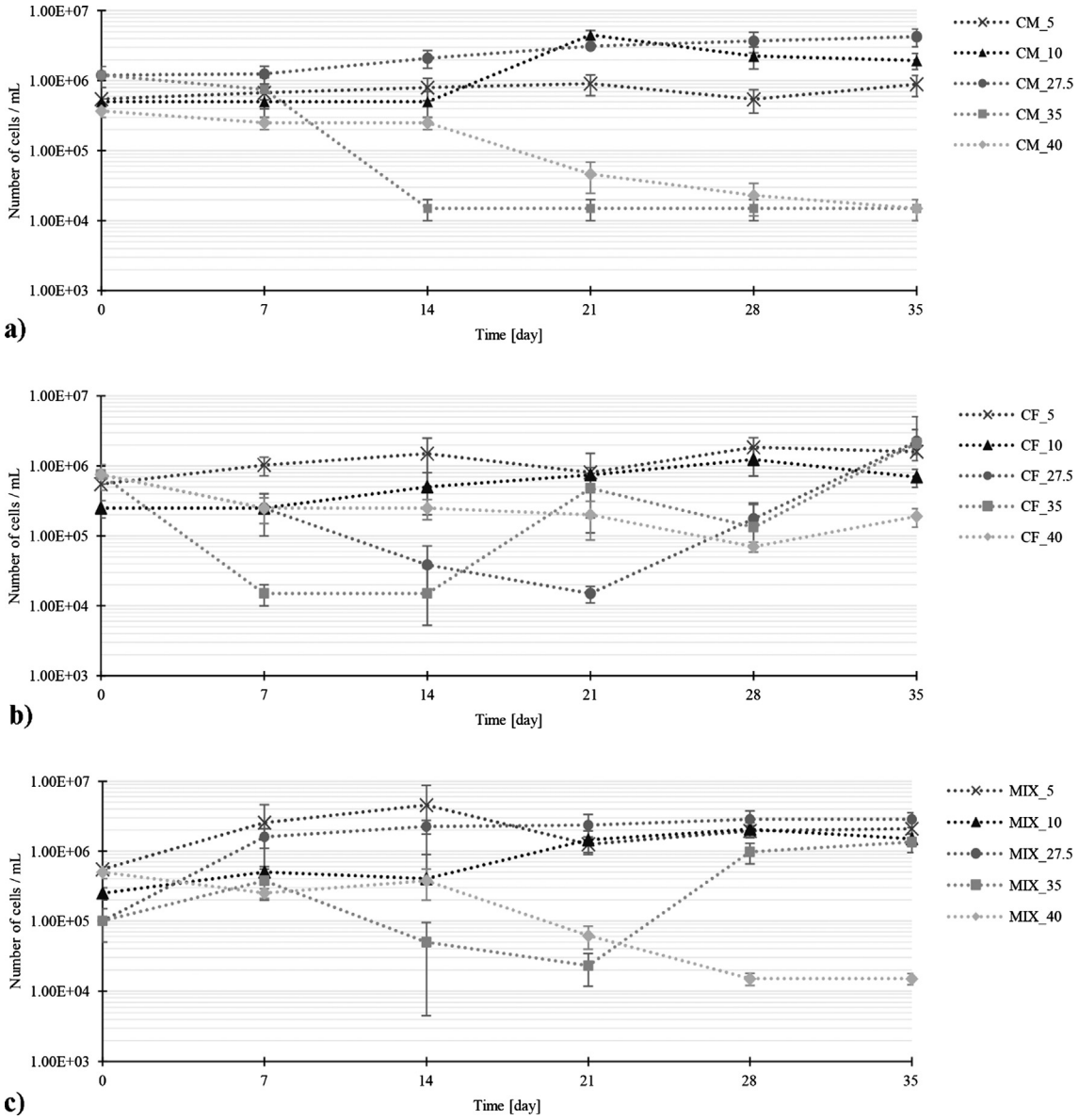

Fig. 2. Growth curves of: a) *Chlorella mirabilis* CM; b) *Chroococcidiopsis fissurarum* CF; c) mixed culture MIX. The growth of the microbial species was tested at different temperatures, namely: 5 °C, 10 °C, 27.5 °C, 35 °C, and 40 °C.

in mixed microbial cultures, a competition for nutrients and space can occur, thus leading to a possible prevalence of one species over another [59].

In order to assure the presence of both the two mixed phototrophic microorganisms in the building materials to be tested, the growth temperatures of accelerated tests were chosen following the results of this test. Thus, T = 10 °C and T = 27.5 °C were selected, as the most suitable temperatures to allow the growth of both *Chlorella mirabilis* and *Chroococcidiopsis fissurarum*. The chosen temperatures correspond to what is reported into the available literature [35,36].

3.3. Results of accelerated growth tests under different constant RH

Fig. 3(a–c) shows the total colour difference ΔE of the fired brick surfaces exposed to the three different RHs. Samples AR showed no significant differences under the three tested RHs: average total colour variations after the 36th week were all just lower than 1. Samples B showed a lower average colour variation ΔE than samples AR, thus under the limit of human perception. Lastly, average colour variations higher than 1 (but lower than 2) were registered on samples CR from 9th week onward when exposed to RH_3 = 98%, while an average $\Delta E < 1$ was measured under RH_1 and RH_2. In general, at the end of the 36th week, most of the samples showed an average total colour difference ΔE lower than 1, and only few samples exceeded this value with a chromatic variation noticeable only after a very deep observation [52]. In these cases, a $\Delta E < 2$ was always observed, and the red/green difference Δa^*, investigated to check a possible chromatic change to green due to the presence of chlorophyll, gave always positive values on average (Fig. 3(d)).

Therefore, at RH $\leq$ 98% no (qualitative) signs of algae growth seems to be present.

Digital Image Analysis results confirmed the previous qualitative colorimetric analysis. Measurements are not reported, since the covered area during all the tests was always equal to 0 for each sample. This quantitatively confirmed that the exposition to RH $\leq$ 98% does not seem to allow algae growth on the tested fired brick samples.

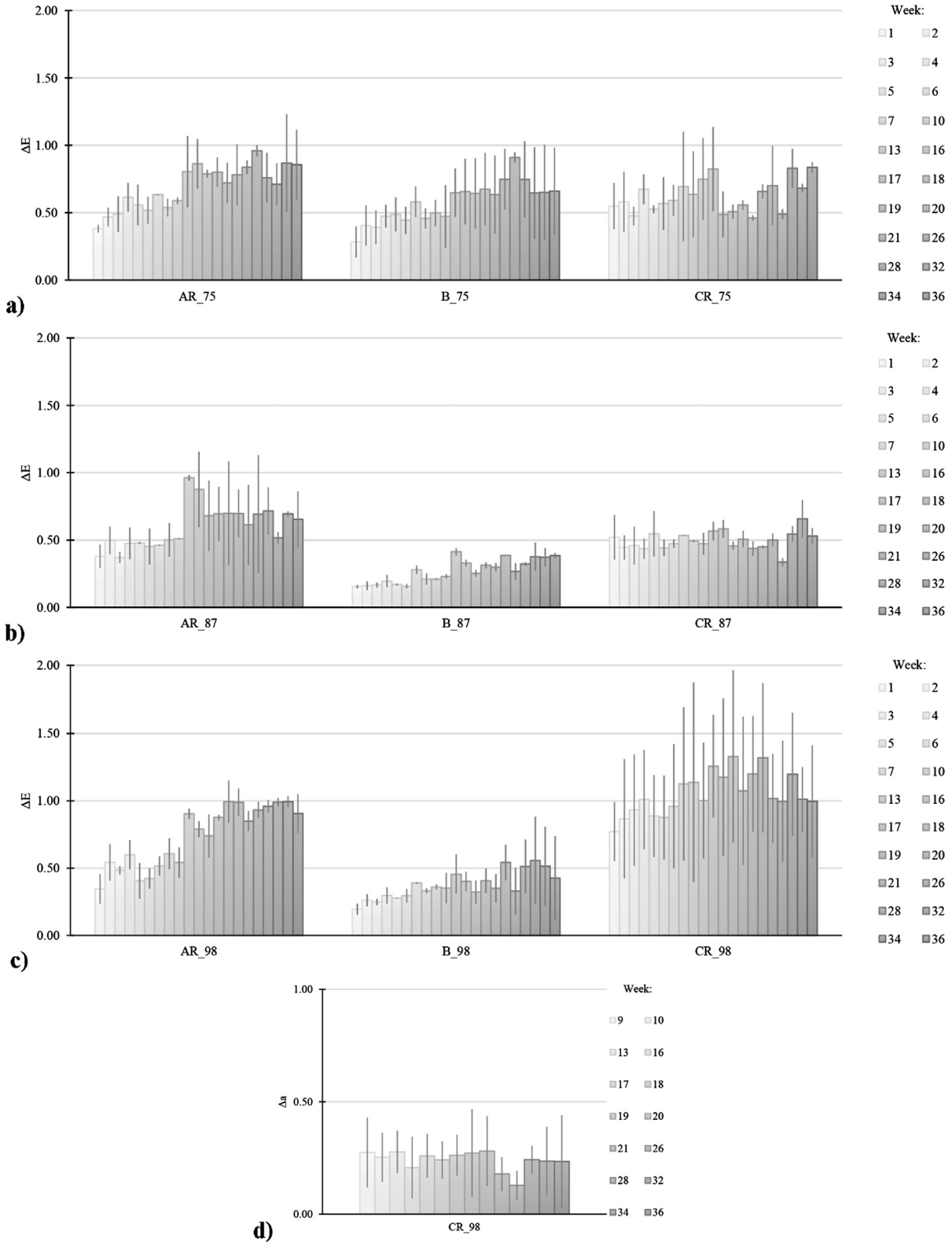

Fig. 3. Total colour difference (ΔE) of sample AR, B and CR: a) RH_1 = 75%; b) RH_2 = 87%; c) RH_3 = 98%. Red/green difference Δa*: d) sample CR_98. Results are reported weekly (in grey scale for a total of 36 weeks); vertical line bars indicate standard deviations.

Hence, considering results from both qualitative (chromatic variation) and quantitative analysis (DIA), and assuming RH = 98% as a safety limit to be not overcome, from an engineering point of view, it can be said that algae biofouling cannot occur on fired brick surfaces at RH < 98%. This is independent on substrate properties such as total porosity and roughness.

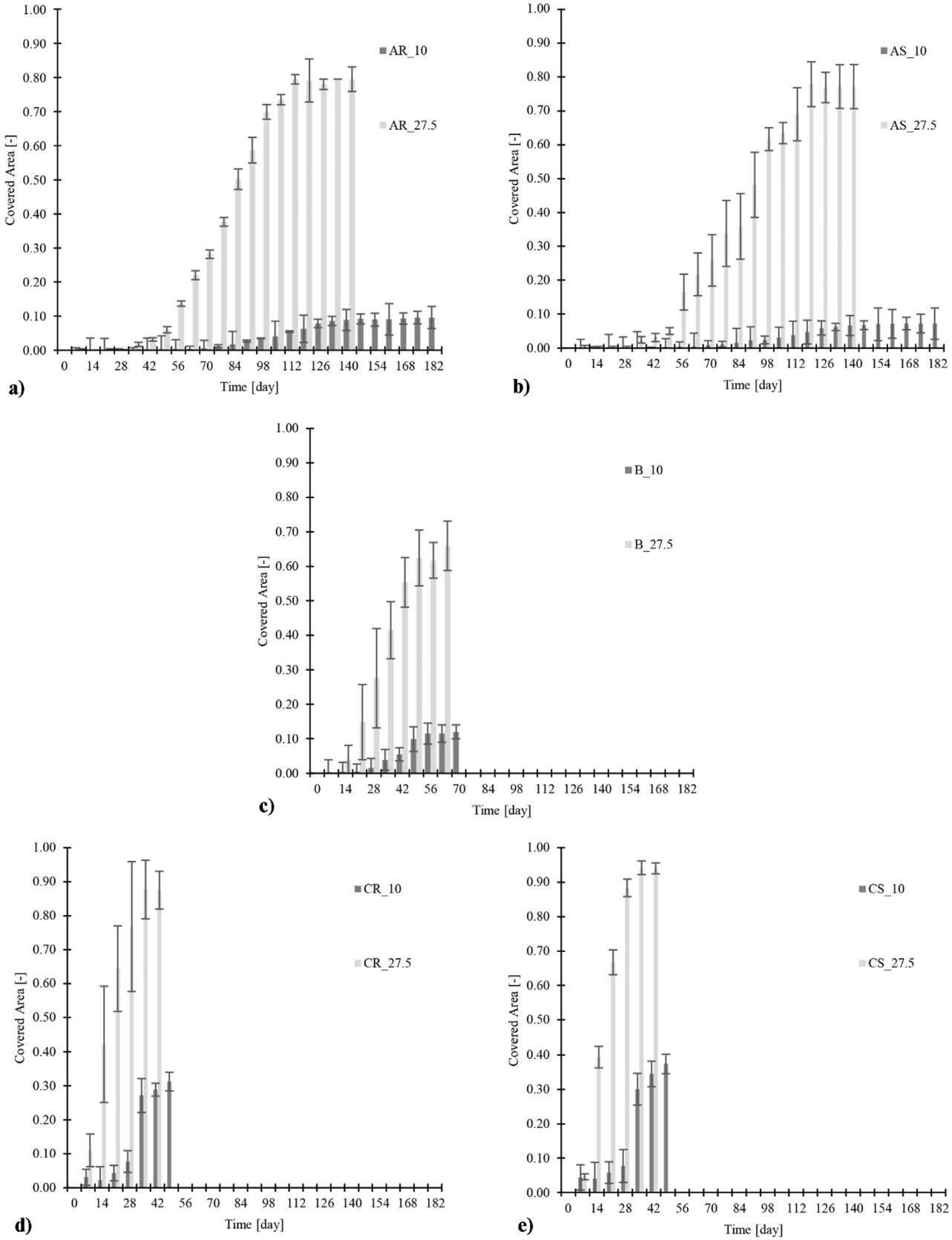

Fig. 4. Average covered area of fired brick: a) samples AR; b) samples AS; c) samples B; d) samples CR; e) samples CS. Results for T = 10 °C are reported in black, results for T = 27.5 °C are reported in grey, vertical line bars indicate standard deviation.

3.4. Results of accelerated growth tests under different constant temperatures

Fig. 4 reports the results obtained from accelerated run-off tests on fired brick materials. Samples AR and AS showed similar growth processes after the exposure under 27.5 °C (Fig. 4a,b). Quasi-null growth rates were observed until the 27th day, and the maximum covered areas in both cases reached about 80% after 140 days. Considering results from tests at T = 10 °C, biofouling was significantly affected by the lower temperature: the measured covered areas were less than 10% of the total area after 182 days (Fig. 4a,b). Samples B tested at T = 27.5 °C were covered on average only up to 65% of the total area after 63 days, while they reached on average 12% of coverage after about 70 days at T = 10 °C (Fig. 4c). Considering samples C, both rough (CR) and smoothed (CS), they showed a percentage of covered area equal to 90% in tests at T = 27.5 °C (Fig. 4d, e). After 42 days the trend of the registered data reached the stagnation phase. On the contrary, at T = 10 °C (Fig. 4d,e), CR and CS samples showed on average the maximum algal coverage only for the 35% of the total area.

Hence, comparing the measurements of the two accelerated tests, it is possible to assess that the covered area decreased when the samples were exposed to a colder temperature (T = 10 °C). Moreover, the lower temperature extended the growth process time by 15–30%.

3.5. The influence of substrate properties on algae growth under constant temperatures

Fig. 5(a) reports the number of days until the stagnation phase was reached in each sample. No significant differences were observed between rough and smoothed samples. However, it is evident how a high porous brick, as samples C (44.09%, Table 1), accelerated the biofouling process in terms of time, if compared to a low porous brick, as samples A (19.24%, Table 1). Porosity also influenced the effect of temperature. The duration of the biofouling process on high porous samples (C) was increased by 15% if exposed at a colder temperature (10 °C). On the contrary, for less porous substrate (samples A) the time process was increased by 30%. Thus, the effect of temperature was enhanced on sample characterized by low porosity.

The effect of roughness on the average covered area at the end of the tests is reported in Fig. 5(b). The higher roughness values of samples CS and CR (6.60 μm and 7.60 μm, Table 1) favoured the algae growth, if compared to sample B, characterized by a lower roughness (2.95 μm, Table 1). Roughness influenced the effect of temperature, too. On sample C the average algal coverage decreased by 60%, while on low rough samples (B) it was decreased by 80%.

Finally, it can be pointed out that porosity strongly influences the rate of biofouling process: a higher value of porosity corresponds to a faster algae growth. At the same time, roughness seems to affect the covered area reached at the end of the biofouling: the percentage of algal coverage has an increasing trend from smoother to rougher surfaces. Moreover, the biofouling is significantly reduced by the effect of a substrate characterized by low porosity and/or low roughness combined with a low temperature.

SEM micrographs (Fig. 6) show the growth of algae and cyanobacteria on the two tested samples. It is well evident that the biomass accumulates along and across the micro-cavities (pores) and clings to the micro-asperities (roughness) of the substrate. Thus, it is possible to suppose that pores facilitate the establishment of algae and cyanobacteria as biofouling, as well as an irregular surface (roughness) their spread.

This seems to confirm what has previously been elucidated. That is, on the one hand, the lower the porosity the higher the stagnation time, since biofouling needs a greater time to cover the greater distance (on average) from one pore to another. On the other hand, the higher the roughness the higher the covered area, since the presence of micro-indentations on the material surface favours the mechanical anchorage of the microbial cells, which progressively proliferated and grew, forming filamentous algal biomass. Clearly, a high porosity joined to a high roughness causes low stagnation times, as well as a great spread of the tested microorganisms, thus leading to a high covered area.

3.6. Modelling of the experimental results

The modified Avrami's curves *X(t)*, analytically modelled by the parameters obtained from the accelerated growth tests, under T = 27.5 °C and T = 10 °C, are reported in Fig. 7. For all the samples, the curves on average tend to slightly underestimate the initial part of experimental results and, on the contrary, to slightly overestimate the growth process before the last phase of stagnation. However, the analytical values were generally included within the average experimental values and their standard deviation.

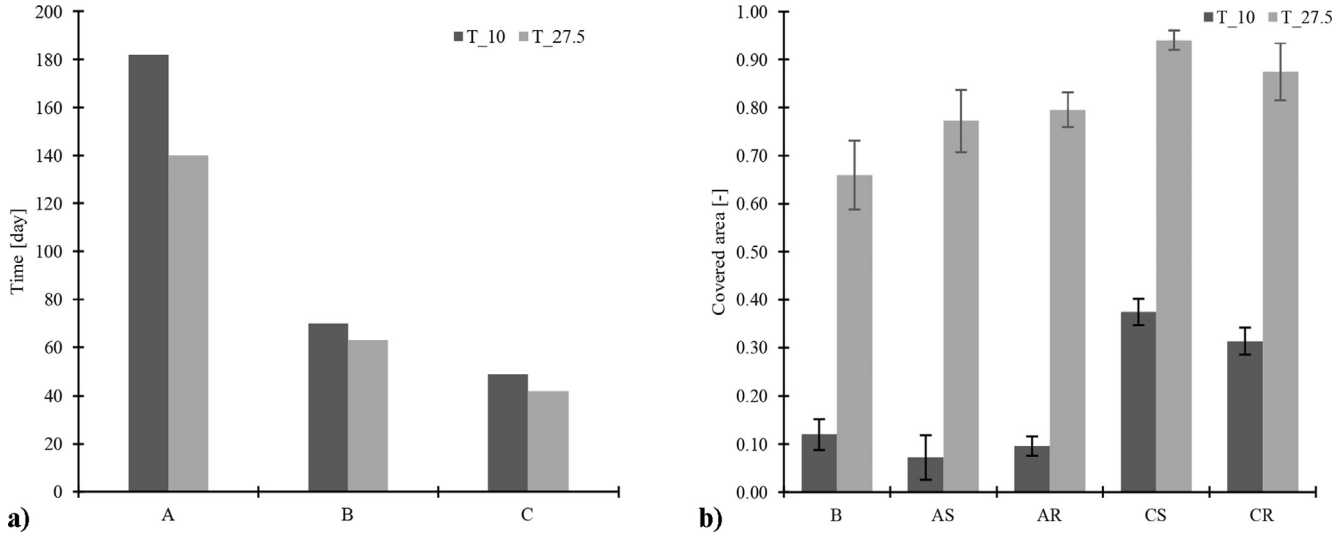

Fig. 5. a) Duration of the accelerated biofouling process on different fired bricks classified by porosity; b) Average covered area at the end of the tests on fired bricks classified by roughness.

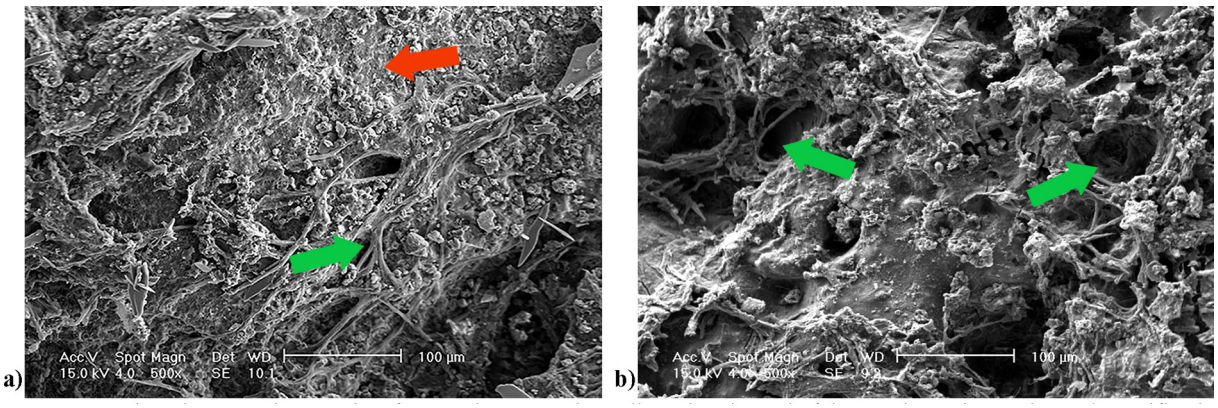

Fig. 6. Scanning electron micrographs of AR and CR samples collected at the end of the accelerated growth test (magnification 500x): a) AR sample, algae and cyanobacteria (green arrow) grow on pores following the micro-asperities of the substrate but leave the smoother surface free (red arrow); b) CR sample, algae and cyanobacteria grow inside pore cavities (green arrow) and adhere to the many surface irregularities. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

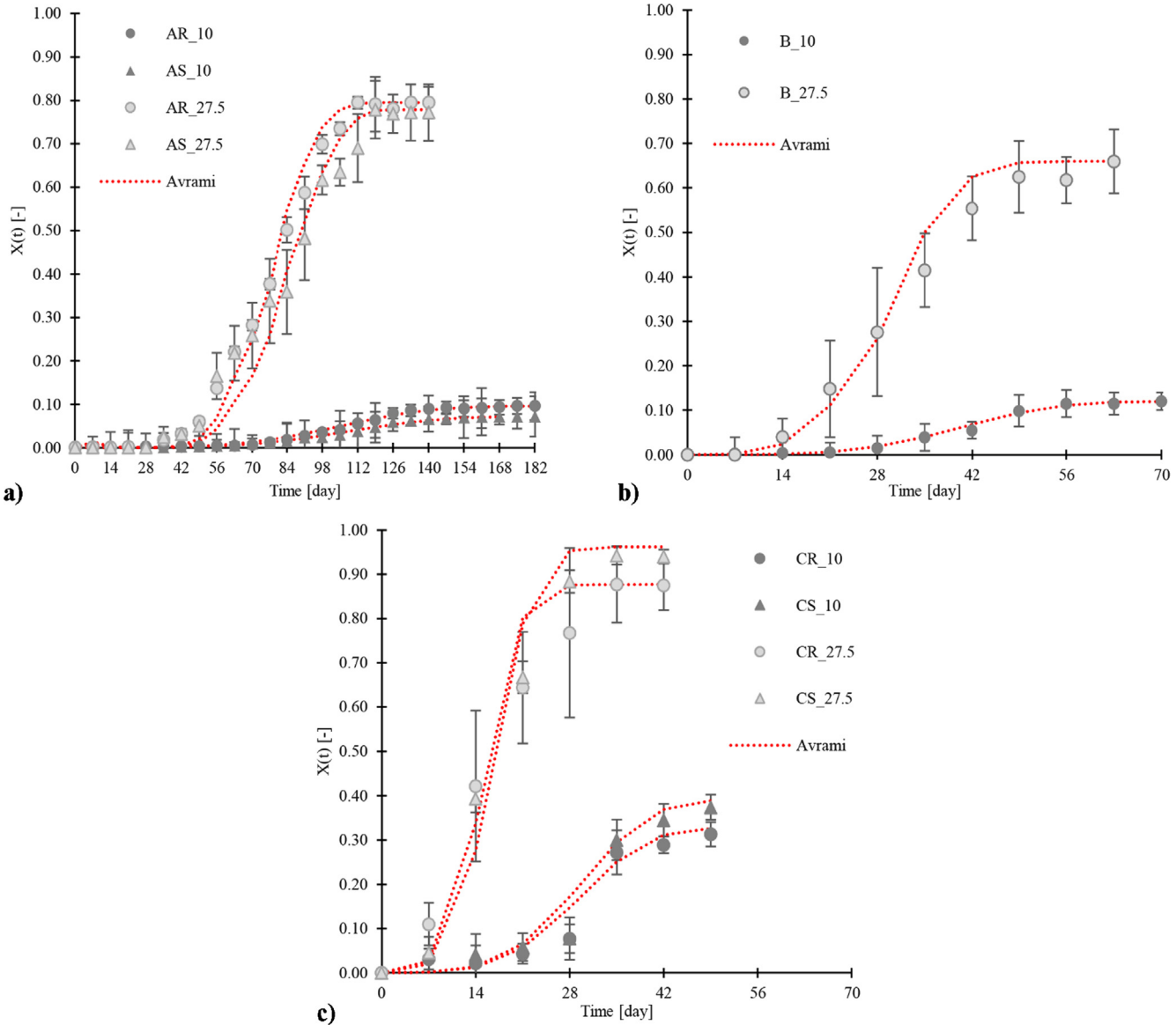

Fig. 7. Overlapping of the modified Avrami's curve to experimental data: a) AR and AS samples; b) B sample; c) CR and CS sample. Results for T = 10 °C are reported in black, results for T = 27.5 °C are reported in grey, red dotted line indicate the modified Avrami's curve. Vertical line bars indicate standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Calculated Avrami's parameter.

Sample	Temperature tested [°C]	A_c/A_t [%]	K [–]	t_1 [day]	R [%]
AR	27.5	79.5	$1.10 \cdot 10^{-7}$	27	5
AS		77.9	$7.00 \cdot 10^{-8}$	27	10
B		66.0	$9.47 \cdot 10^{-7}$	0	9
CR		87.7	$1.27 \cdot 10^{-5}$	0	10
CS		94.2	$8.80 \cdot 10^{-6}$	0	8
AR	10	9.6	$5.94 \cdot 10^{-9}$	27	7
AS		7.2	$5.32 \cdot 10^{-9}$	27	4
B		12.0	$2.68 \cdot 10^{-7}$	0	7
CR		32.7	$9.74 \cdot 10^{-7}$	0	12
CS		35.2	$9.40 \cdot 10^{-7}$	0	12

Hence, it can be assessed that the analytical curves well model the experimental measurements.

Table 2 shows the parameters that were used for the analytical calculation of $X(t)$ [27,28]. Considering the temperature of 27.5 °C, the ratio A_c/A_t ranged on average from 66% for samples B, to the maximum of 94.2% for samples CS. K values were significantly different from each other, and ranged from $7.00 \cdot 10^{-8}$ for samples AS to $1.27 \cdot 10^{-5}$ for samples CR. Only for samples A, for both rough and smoothed surface, a latency time t_1 was observed and it was equal to 27 days. Considering the temperature of 10 °C, the maximum covered area A_c/A_t was 35.2% on samples CS. K values ranged from $9.74 \cdot 10^{-7}$ for samples CS to $5.32 \cdot 10^{-9}$ for samples AS. Latency time t_1 was the same as for temperature of 27.5 °C. Therefore, the parameters A_c/A_t and K had a severe decrease when related to a colder temperature: except for samples B, growth rate K diminished more than 10 times and A_c/A_t was reduced up to 35%.

Finally, in Table 2 the R factor values are reported. They indicate, in percentage, how much the analytical curves differ from the experimental data. Values vary between 5% and 10% for the temperature of 27.5 °C and between 4% and 12% for the colder temperature of 10 °C. Thus, it can be reasonably stated that the modified Avrami's model is adequate to describe algal growth also for temperatures lower than the optimal one.

4. Conclusion

Up to now, researches on growth of microalgae and cyanobacteria on building materials, with particular reference to fired bricks, have shown that the substrate properties, such as total porosity and roughness, play an important role in biofouling. However, all the available studies referred to tests usually performed under optimal conditions of temperature and relative humidity, without considering the important role of different environmental conditions, that has taken into account in this work. A green alga (*Chlorella mirabilis*) and a cyanobacterium (*Chroococcidiopsis fissurarum*) were tested, since they can be commonly found on building façades in European countries.

The results from preliminary growth tests at different temperatures showed that for temperatures higher than 35 °C the presence of the green alga *Chlorella mirabilis* significantly decreased, while the cyanobacterium had a variable growth trend regardless of the environmental temperature.

Concerning the tests aimed at evaluating the effect of relative humidity, the colorimetric variations detected on samples' surfaces were generally lower than the perceptible threshold for human eye. Moreover, considering the red/green variations it was shown a mild colour change, that was not linked to the appearance of algae. Quantitative analysis (DIA) confirmed this result, since no covered area by algae was detected on any sample at the tested RHs. Thus, experimental results showed that there was no growth at the tested relative humidities, and, from an engineering standpoint, RH < 98% could be assumed as a safety limit against algae growth on fired bricks, independently from substrate properties such as total porosity or roughness. Therefore, it can be reasonably assumed that the phenomenon of growth can only occur in the presence of free water on the material surface. Some more experimental tests are however needed to confirm these results in terms of different range of porosity and roughness.

From the accelerated growth tests conducted under T = 10 °C and T = 27.5 °C, the algal biofouling was highly influenced by the temperature conditions. A colder temperature slightly reduced the rate of the biofouling process and the total covered area at the end of the test significantly decreased (by about 75%) if compared to the exposure under optimal temperature. From experimental results, it was confirmed the role of the substrate on algae growth: high porosity and high roughness favoured the colonization, by influencing it in terms of time and covered area at the end of the process. In this way, pores seem to facilitate the settlement of the biofouling, as well as an irregular surface (roughness) its spread. Thus, the lower the porosity the higher the stagnation time, because the microorganisms need, on average, a greater time to cover the greater distance from one pore to another. As well as, the higher the roughness the higher the covered area, because the micro-asperities of the substrate favour the mechanical anchorage of the microorganisms.

Finally, it was shown that the modified Avrami's model was able to predict algae growth, not only for optimal temperature conditions, but also for colder ones. However, a failure model about algae growth is not available yet. A model which explicitly includes parameters to takes into account environmental conditions (such as temperature and relative humidity) during the time and the properties of the substrate is thus needed. To this aim investigations under further temperatures should be performed and more different materials should be tested to increase the knowledge on the complex phenomenon of algae growth and the consequent deterioration effect on building materials.

Conflicts of interest

None.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 637268.

Authors are grateful to Mr. Pierluigi Rondoni of the University Center for the Electron Microscopy (CUME) of the University of Perugia (Italy) for his valuable contribution in realizing the photos using the scanning electron microscope (SEM).

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