Article

On the Modelling of Algal Biofouling Growth on Nano-TiO₂ Coated and Uncoated Limestones and Sandstones

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Abstract: Algal biofouling on archaeological and historic materials, as well as in modern building façade, is a common phenomenon that occurs when microorganisms of various nature adhere to the material, forming biological stains and patinas. It can significantly deteriorate the aesthetic and even mechanical quality of historic and archaeological artifacts. Thus, predicting the colonization progress of algae on treated and untreated materials can be helpful to establish appropriate schedules and methods of maintenance. In this way, the aim of this research was to modelize the algal colonization on nano-TiO₂ coated and uncoated stone surfaces, usually found in historic and archaeological artifacts, by following Avrami’s theory. Particular attention was paid on correlating the model with some properties of the substrate, like roughness and porosity. Biofouling was tested on two sandstones and three limestone with different intrinsic characteristics (porosity, roughness) by means of an accelerated lab-scale test. A suspension of green alga *Chlorella mirabilis* and cyanobacteria *Chroococcidiopsis fissurarum* was used as biofouling. Digital image analysis was carried out in order to find the attachment rate and the growth of algal spots. Results show that the attachment specific rate increased linearly with time, and the assumption of a constant growth rate was acceptable. A good agreement between the simulation and the experimental results was obtained with a maximum error of 0.59%.

Keywords: biofouling; stones; modeling; durability; nanotechnology; titania; porosity; roughness; Avrami; nano-coating

1. Introduction

Algal biofouling is the accumulation of microorganisms on wetted surfaces, and it also widely involves the building construction field because the attachment of biotic organisms on the building components (especially those exposed to weathering) can compromise the aesthetic quality of the entire buildings [1,2]. This also holds for archaeological and historic artifacts, where algae may also deteriorate their mechanical properties [3,4]. Many studies are present in the literature about algal biofouling process on building materials like stones [2,3], bricks [5], ETICS (External Thermal Insulation Composite System) [6], concrete [7,8] and mortars [9–11]. Some of these studies have deepened this issue by found correlations between the adhesion of microorganisms and some characteristics of the substrates, mainly roughness and porosity [2,5,6,9].

At the same time, many research papers have investigated on the most effective and cheaper solutions to limit (or solve) this problem and some recent articles have found a good candidate in TiO₂ nano-coatings, because of their biocide power under UV irradiation, their durability and affordable costs [12–17] as well as because they have self-cleaning and depolluting effects [18–21], too. They seemed to be able to slow down algal biofouling when applied on low porous materials with
smooth surfaces [22], even if algal growth is not inhibit on the substrate [13,23–25] and to facilitate its removal on some substrates through the formation of a superficial water film [5].

Tests on algal biofouling prevention were set under controlled climatic conditions, and they regarded specific building materials. Thus, laboratory tests can be transferred only to real conditions with the same climatic conditions and to artifacts made by the same tested materials. It turns out that a generalization of the problem to real case studies is very limited. Literature shows that a mathematical generalization of the problem is thus occurring, and the number of papers on the argument is raising [26–31]. These research articles focus on the comparison between 1D, 2D and 3D models independently from the typology of the substrates, and they did not consider the influence of porosity or roughness.

The literature also shows that studies aimed at the modeling of algal biofouling on building materials are very limited, due to the complex nature of the problem. The modeling of biofouling involves a complex non-linear treatment because of the unpredictable behavior of microorganisms and their relationship with building materials and environment conditions.

Biofouling on solid substrata was recently proposed to predict the growth of microorganisms on the surface of monuments, and a nonlinear hyperbolic–elliptic partial differential equation was used [32–34]. The dynamic biofilm growth in porous media at a microscopic scale was developed by implementing a growth of the biofilm in irregular domains by considering the thickness of the substrates as an independent variable [35].

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The biofouling process on a building material was numerically simulated by mean of the Avrami’s theory [36–38]. The growth of biofilms on the surface was described in terms of number of algal spots and their growth by time. This approach allowed to correctly simulate the biofouling on mortar surfaces under accelerated laboratory growth conditions [39]. Recent studies propose a machine learning method based on the least squares support vector regression (LS-SVR) for modelling the growth time of the green alga Klebsormidium flaccidum on mortar surfaces [40,41]. They demonstrate that the hybrid model is a promising tool for assisting the decision-making process in building maintenance planning.

The diffusion of the Avrami’s model and of the LS-SVR model to predict algal growth can be attributed to an appreciable learning generalization and a very fast computation. These characteristics are important from an engineering point of view because they allow to consider large surfaces (i.e., a masonry wall), and many materials.

In this way, the aim of this paper was to model the algal colonization on nano-
TiO\textsubscript{2} coated and uncoated stone surfaces, usually found in archaeologic and historic artifacts, by following Avrami’s theory, by paying attention on properties of the substrate like roughness and porosity, due to their strong influence on algal growth, as previously said. No mineralogical composition was taken into account because nutrient for algae was artificially provided by the Bold’s Basal Medium (BBM) (ASTM D5589) [42] solution during the accelerated growth test. The nutrient by the mineral inside the stone is thus negligible if compared to the one provided by the BBM.

This can be helpful to establish appropriate schedules and methods of maintenance.

2. Materials and Methods

2.1. Tested Materials and Nano-Treatment Application

In this paper, two types of sandstone, and three types of limestone, usually found in Cultural Heritage, were tested. Six prismatic specimens were prepared for each group, having an area of 80 × 80 mm\textsuperscript{2} and a thickness of 30 mm.

Initial characterization of the tested stone provided the values of porosity and roughness. Total porosity ($p$), and average pore diameter ($d$) were measured by a mercury intrusion porosimeter (Autopore III, Micromeritics, Norcross, GA, USA) following ASTM D4404 [43] standard procedure. A Diavite DH-5 rugosimeter (Diavite, Bülach, Switzerland) was used to measure the roughness of
the surface on eight locations per each specimen. Then, the average surface roughness ($R_a$) and the geometric average height of roughness irregularities ($R_q$) were assessed according to UNI EN ISO 4287 [44].

Table 1 reports the main intrinsic characteristics of each tested specimens.

### Table 1. Classification and intrinsic characteristics of tested stones. Six specimens for each group were tested. Standard deviations were reported ($\pm$).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Stone Typology</th>
<th>$p$ (%)</th>
<th>$d$ (µm)</th>
<th>$R_a$ (µm)</th>
<th>$R_q$ (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Sandstone</td>
<td>7.74</td>
<td>0.08</td>
<td>7.6 ± 0.6</td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td>A2</td>
<td>Sandstone</td>
<td>4.52</td>
<td>0.05</td>
<td>7.9 ± 0.4</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td>C1</td>
<td>Limestone</td>
<td>8.54</td>
<td>0.05</td>
<td>2.6 ± 0.5</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>C2</td>
<td>Limestone</td>
<td>18.17</td>
<td>0.09</td>
<td>2.6 ± 0.2</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>C3</td>
<td>Limestone</td>
<td>7.54</td>
<td>0.06</td>
<td>2.0 ± 0.1</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

Three specimens for each group were treated with a TiO$_2$ aqueous solution composed by 1% (w/v), while the other three remained untreated and used as controls. This percentage was chosen in order to avoid chromatic variations of the substrate. Besides, water was used as solvent so as to reduce the risk of exposition of hazardous materials and to eliminate the chemical action on stones. More details can be found in [22].

In this paper, a commercial TiO$_2$ aqueous solution was used. It was supplied by Salentec S.r.l. (Lecce, Italy), and it was characterized by very fine anatase crystals with an average diameter of 4 nm as estimated by XRD patterns elaboration.

Nano-treatment was manually applied by an air gun from a distance of about 250 mm. Treated specimens were identified with a “T” after the ID, while controls were named with a “U” at the end of the acronym.

#### 2.2. Accelerated Biofouling Test

Experimental data necessary for the computation of Avrami’s equation were supplied by [22], where an accelerated algal growth lab-scale test was performed by following [13,23–25,45]. A brief summary of the experimental set-up is however reported in the following. A suspension of *Chlorella mirabilis* and *Chroococcidiopsis fissurarum* mixed culture was sprinkled on the surface of each specimen inside a glass chamber (100 × 40 × 53 cm$^3$) under controlled climatic conditions (Figure 1). The assembled system was put in a dark room to avoid the influence of external environment in terms of light, temperature and humidity.

![Figure 1. Test apparatus for accelerated biofouling test.](image-url)
A heater maintained the temperature of the suspension at 24 °C and two wave pumps agitated the algal culture to avoid sedimentation of cells and to guarantee the ideal conditions for algal growth. Illumination was provided by two neon lamps that simulate daylight (1500 lux) for the photosynthesis of algae, while two UV lamps guaranteed 8 W/m² on the surface of treated specimens to activate photocatalytic power of TiO₂. Relative humidity (RH) in the chamber was 80%, but it reached 90% during water sprinkling.

Algal suspension was sprinkled for 6 h/day with a run/off cycle of 15 min thanks to two sprinkling rails made of PVC tubes with three 2 mm-holes installed 20 mm above the surface of specimens.

Since the Avrami’s equation is based on the number of algal spot present on the surface and their dimension through time, in this paper digital image analysis (DIA) was used to carry out a particle analysis for each specimen. Each specimen was digitized weekly by an office plane scanner at a 600 dpi resolution. Particle analysis was conducted by ImageJ software (v1.51j8) that is able to obtain the number of algal spot on the stone specimen, their dimension and the percentage of the covered area by microorganisms through time.

Accelerated growth test was stopped when all specimens reached their maximum coverage and maintained it after minimum three subsequent measurements defining a stabilization point.

2.3. Numerical Modelling

This paper adopts the Avrami’s model previously proposed in literature in case of full algal coverage [39] and subsequently modified to take into account a partial coverage, as in case of nano-treated specimens [45]. Thus, two main equations were considered to calculate the biofouling area \( X(t) \) as a function of time: Equation (1) was used to describe a full coverage biofouling, while Equation (2) was used to describe a non-complete biofouling.

\[
X(t) = 1 - e^{-K(t-t_1)^n}
\]  
\[
X(t) = \left(1 - e^{-K(t-t_1)^n}\right) \times \frac{A_c}{A_t}
\]

where \( K \) is a constant of the material depending on the rate of the nucleation of new particles, and the specific growth rate constant, \( t_1 \) is the latency time corresponding to the first comparison of algal spot, and \( n \) is the Avrami’s exponent calculated as in Equation (3). \( A_c \) is the area covered by algae at the end of the accelerated growth test, and \( A_t \) is the total area of the specimen.

\[
n = q + 3
\]

The \( q \) parameter derives from the general equation of growth (Equation (4)) that defines the number of algal spot at time \( t \) per unit area (spot/µm²):

\[
\frac{dy}{dt} = k_y(p, R_a) \times (t - t_1)^q
\]

where \( k_y \) is the specific attachment rate constant (spot/µm²-day²), and it corresponds to the slope of the regression line of the general equation growth. Since Equation (4) was linear in all the tested cases, \( q \) must be equal to one in order to have a first order polynomial equation.

Operatively, the first step consisted in the count of algal spots during time by ImageJ, and to obtain \( k_y \) by linear regression. The spots were counted until an extension of biofouling of about 15% because after this limit algal spots join and the particle analysis was distorted.
Since \( X(t) \) can be determined experimentally by ImageJ, the value of \( K \) can be determined by least square methods knowing \( k_g \) and \( A(q) \) (Equation (5)).

\[
A = \frac{2}{(q + 1)(q + 2)(q + 3)}
\]  

(5)

During DIA for the count of algal spots, ImageJ software provides the experimental parameter \( k_c \) that represents the dimensions of algal stain during time.

By knowing \( K \) (determined by the least square method) and \( k_g \) (determined by DIA), it was possible to derive the analytical \( k_c \) by knowing the Avrami’s equation of \( K \) (Equation (6)):

\[
K = A \cdot k_g \cdot k_c^2
\]

(6)

thus, \( k_c \) was equal to:

\[
k_c = \sqrt{\frac{K}{A \times k_g}}
\]

(7)

This procedure allowed the comparison between analytical and experimental \( k_c \) that is a first validation of the model. A deeper validation was performed calculating a confidential \( R \) factor (Equation (8)) that describe the gap between experimental and numerical curve.

\[
R = \frac{\sqrt{\frac{\sum_{t=1}^{m} (X_{st} - X_{et})^2}{\sum_{t=1}^{m} X_{et}^2}} \times 100}{\sum_{t=1}^{m} X_{et}^2}
\]

(8)

where \( X_{st} \) is the simulated colonization rate and \( X_{et} \) represents the experimental colonization rate.

2.4. Statistical Analysis

In this paper a one-way analysis of variance (ANOVA) was carried out to interpret the significance of results and to find real difference between treated and untreated specimens. In detail ANOVA was carried out to compare the values of \( X(t) \), \( k_g \), and \( k_c \) between untreated specimens and treated ones. In addition, statistical analysis was carried out to construe the difference between analytical and experimental \( k_c \). The Tukey–Kramer honestly significant difference (HSD) was adopted as statistical model by fixing a significance of differences at \( p \)-value \( p < 0.05 \).

Thus, when ANOVA result is lower than 0.05, a significant difference is present between the compared groups, otherwise the difference is negligible. Statistical results were reported in the paper in terms of significance, and by indicating each \( p \)-value.

At the end of the analytical procedure, the reliability of the model was statistically evaluated by plotting the correlation between experimental \( X(t) \) and analytical \( X(t) \), and by finding the correlation factor \( (R^2) \).

3. Results and Discussion

In this section, the values of the parameters explained in Section 2 were highlighted starting from the extension of algal coverage experimentally evaluated by DIA. Then, all the Avrami’s parameters were reported, and the overlapping between experimental and analytical \( X(t) \) curve was evidenced at the end of the section.

3.1. Algal Coverage \( X(t) \) from DIA

Results obtained by DIA were reported in Figure 2. They shows the experimental algal coverage \( X(t) \) during time.
Figure 2 shows no significant difference between treated specimens and control ones. In case of C1 treated specimens biofouling was even larger than untreated stones. This is not surprising. In fact, when TiO$_2$ is obscured by algae, it loses the photocatalytic properties because UV light does not reach titania particles, and the behavior of treated specimens is practically the same of untreated ones [22]. This is confirmed from the overlapping of the vertical bars (standard deviations) in Figure 2, which indicates the substantially equal behavior between treated and untreated specimens.

The anti-biofouling effect of TiO$_2$ is quite visible only in A1 and C3 from time 14 to 21 and from time 14 to 42, respectively, indeed untreated specimens shown higher values of $X(t)$ if compared with nano-treated stones.

![Graphs showing algal coverage](image)

**Figure 2.** Algal coverage evaluated by digital image analysis (DIA): (a) A1 specimen; (b) A2 specimen; (c) C1 specimen; (d) C2 specimen; (e) C3 specimen. Treated stones (marked with T) and controls (marked with U) of each group were reported in each plot. Vertical bars indicate standard deviation.

### 3.2. General Equation of Growth

The general equations of growth (4), determined as described in Section 2.3, were resumed in Figure 3. Plots show the variation of the number of algal spot in time, and they report the equations of growth. The experimental $k_g$ values are the angular coefficients of equations in Figure 3.
The values of $k_g$ were statistically the same for treated and untreated stones except for C3 specimens ($p$-value between $k_g$ of C3U and C3T is 0.049).

These first results confirm experimental findings, indeed the model shows that TiO$_2$ nano-coating was generally unable to prevent biofouling, but only to delay it, in only one case from a statistical standpoint.

A quite efficiency of the nano-treatment in C3 stone was notable, in fact this specimen shows a different latency time between treated (14 days) and untreated surface (7 days). The effect of TiO$_2$ on C3 can be attributed to the physical properties of the stone, indeed C3 is the one with the lowest porosity and roughness, this helps the photocatalytic action of TiO$_2$ toward biofouling process as reported in literature [23,24,45,46].

![Figure 3](image_url). Number of algal spots with standard deviations (±): (a) A1 specimen; (b) A2 specimen; (c) C1 specimen; (d) C2 specimen; (e) C3 specimen. Regression lines and equations are reported for treated specimens (solid line), and untreated ones (dashed line).
3.3. Parameters of Avrami’s Equation

In this section the remaining parameters of Avrami’s equation were resumed and discussed in relation to the physical properties of the substrates and to the nano-treatment efficiency. Furthermore, the validity of the model was showed by overlapping the analytical curves with the experimental ones.

Table 2 shows the analytical values of latency time ($t_1$) that are also visible in plots in Figure 3, and it corresponds to the time where linear regression intersect x-axis. Standard deviations of $t_1$ was determined by considering the results of three specimens for each group. $t_1$ has the same value for both treated and untreated specimens except in case of C3 where it was 6.56 for C3U and it was 12.80 for C3T. This confirms a certain efficiency of TiO$_2$ when it was applied on low porous stone with a smooth surface. Comparing $t_1$ between treated and untreated specimens of the other groups gives insignificant differences.

A similar trend can be noticed for the other parameters. Indeed, C3T has the lowest value of $k_g$ indicating the adhesion of new algal cells and the comparison of algal stains are slower than in the other specimens.

Besides, the model shows that the nano-treatment was able to slightly slow down the expansion of algal spots in case of A1 and C3 stones because on average the difference between treated and untreated stones of these groups was significant. The $p$-value between A1T and A1U was 0.0201 (for experimental $k_c$) and it was 0.0015 in case of analytical $k_c$. ANOVA analysis declared a $p$-value between C3T and C3U equal to 0.0007 in case of experimental $k_c$ and a value of 0.0015 in case of analytical $k_c$. Statistical analysis between treated specimens and control ones of the other groups confirmed no significant difference.

The same conclusions on $k_c$ can be deduced by considering $K$ value. The groups that showed significant difference between T and U specimens were A1 ($p$-value = 0.0485) and C3 ($p$-value = 0.0118).

$K$ parameter is also able to catch and describe the effect of substrates on biofouling process because it takes into account the effect of $k_g$ and $k_c$ simultaneously [45].

In detail, $k_g$ is mainly influenced by the roughness of the surface, indeed the rougher is a stone the higher is $k_g$ value because the roughness promotes algal adhesion and the formation of new algal spots consequently [23,24]. At the same time, $k_c$ takes into account the effect of porosity because the higher is the porosity, the higher is the amount of water (and nutrients) needed for the growth of algae [23,24].

The effect of the substrates in terms of $K$ values is notable by comparing untreated with treated specimens. Untreated sandstone (A) shows higher values of $K$ than untreated limestone (C), and it reflects the results about roughness. Likewise, treated sandstone (A) shows higher values of $K$ than treated limestone (C).

**Table 2.** Experimental and calculated Avrami’s parameters with standard deviations (±), and confidential $R$ factor. The simulated biofouling was concordant with real colonization when $R$ is at its minimum (or zero).

<table>
<thead>
<tr>
<th>Stone</th>
<th>$t_1$ (day)</th>
<th>$k_g$ ($\times 10^{-10}$) (spot/µm$^2$·day$^2$)</th>
<th>Experimental $k_c$ (µm/day)</th>
<th>Analytical $k_c$ (µm/day)</th>
<th>$K$ ($\times 10^{-6}$) (spot/day$^4$)</th>
<th>$R$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1T</td>
<td>6.56 ± 0.08</td>
<td>191.78 ± 63.69</td>
<td>72.87 ± 2.68</td>
<td>73.05 ± 5.91</td>
<td>9.17 ± 4.35</td>
<td>0.07</td>
</tr>
<tr>
<td>A1U</td>
<td>7.05 ± 0.62</td>
<td>159.90 ± 10.55</td>
<td>122.76 ± 13.07</td>
<td>131.00 ± 4.50</td>
<td>21.87 ± 1.25</td>
<td>0.14</td>
</tr>
<tr>
<td>A2T</td>
<td>6.51 ± 0.10</td>
<td>184.64 ± 67.91</td>
<td>83.06 ± 5.00</td>
<td>83.15 ± 8.55</td>
<td>11.79 ± 5.80</td>
<td>0.21</td>
</tr>
<tr>
<td>A2U</td>
<td>7.28 ± 0.79</td>
<td>213.14 ± 76.05</td>
<td>97.55 ± 7.13</td>
<td>98.59 ± 14.89</td>
<td>20.76 ± 10.44</td>
<td>0.52</td>
</tr>
<tr>
<td>C1T</td>
<td>6.55 ± 0.00</td>
<td>242.71 ± 33.53</td>
<td>58.81 ± 1.46</td>
<td>55.76 ± 3.21</td>
<td>6.10 ± 1.11</td>
<td>0.25</td>
</tr>
<tr>
<td>C1U</td>
<td>6.55 ± 0.00</td>
<td>128.66 ± 26.01</td>
<td>51.25 ± 18.76</td>
<td>56.60 ± 19.72</td>
<td>4.69 ± 3.01</td>
<td>0.30</td>
</tr>
<tr>
<td>C2T</td>
<td>6.50 ± 0.14</td>
<td>104.67 ± 7.52</td>
<td>101.30 ± 9.66</td>
<td>108.21 ± 7.77</td>
<td>9.81 ± 1.26</td>
<td>0.45</td>
</tr>
<tr>
<td>C2U</td>
<td>6.65 ± 0.05</td>
<td>115.62 ± 23.18</td>
<td>111.46 ± 6.66</td>
<td>111.40 ± 9.62</td>
<td>10.98 ± 1.05</td>
<td>0.59</td>
</tr>
<tr>
<td>C3T</td>
<td>12.80 ± 0.53</td>
<td>72.15 ± 2.65</td>
<td>53.69 ± 3.78</td>
<td>59.51 ± 5.49</td>
<td>2.05 ± 0.31</td>
<td>0.18</td>
</tr>
<tr>
<td>C3U</td>
<td>6.56 ± 0.21</td>
<td>110.58 ± 13.35</td>
<td>117.91 ± 5.80</td>
<td>114.37 ± 4.40</td>
<td>11.81 ± 2.20</td>
<td>0.58</td>
</tr>
</tbody>
</table>
3.4. Reliability of the Model

A first interpretation of the reliability of the model can be deduced by $R^2$ in Table 2, indeed all the values are lower than 0.59%, indicating a good agreement between $X_{st}$ and $X_{et}$.

In this section the reliability of the adopted model was showed by reporting the correlation plots between experimental $X(t)$ and analytical $X(t)$ (Figure 4). In addition, each plot shows the correlation factor ($R^2$) of the regression line with the bisector.

Figure 4 shows a very good agreement between $X(t)$ experimentally evaluated and $X(t)$ from the Avrami’s model. The minimum $R^2$ is equal to 0.978 (C3U), denoting that calculated data fit the experimental ones. This can be also appreciated in Figure 5 where the analytical curve is overlapped to the experimental values.

These results about reliability are in line with other results from literature where the same model was applied [39,45].

Results from this paper show that Avrami’s model was able to catch the same phenomenon verified during the experimental test. In detail, it provided analytical information about the efficiency of the nano-treatment, and correlations between physical properties of the substrate, such as porosity and roughness, and its parameters $k_g$ and $k_c$.

![Figure 4](image-url)
Avrami’s theory can be applied to model the colonization of micro-algae on both treated (by nano-TiO$_2$) and untreated sandstone and limestone, typical stones used in historic and archaeological artifacts. In this way, this paper demonstrates that the efficiency of these coatings is strongly dependent on some properties of the substrate. Further details about the influence of porosity and roughness on the biofouling process on natural stone and bricks can be found in literature [23–25,47].

4. Conclusions

TiO$_2$-based nano-coatings have been becoming more and more widespread biocide treatments on historic and archaeological stone artifacts during last years. However, some recent researches have pointed out that the efficiency of these coatings is strongly dependent on some properties of the substrate itself (in particular, its porosity and its roughness). Thus, predicting the colonization progress of algae on treated and untreated stones by taking into account these properties can be helpful to establish appropriate schedules and methods of maintenance. In this way, this paper demonstrates that Avrami’s theory can be applied to model the colonization of micro-algae on both treated (by nano-TiO$_2$) and untreated sandstone and limestone, typical stones used in historic and archaeological artifacts.

**Figure 5.** Overlapping of analytical curves to experimental ones. Confidential R factor was reported for each series: (a) A1 specimens; (b) A2 specimens; (c) C1 specimens; (d) C2 specimens; (e) C3 specimens. Vertical bars indicate standard error.

Since the aim of this paper was the application of Avrami’s theory to build a predictive model for algal biofouling growth, results focused on the interpretation of parameters, and on the reliability of the model. Further details about the influence of porosity and roughness on the biofouling process on natural stone and bricks can be found in literature [23–25,47].
Data set was collected in accelerated growth conditions by the use of digital image analysis technique for the quantification of the analytical parameters.

The model confirms to be adequate to describe the biofouling process, and to show the influence of physical properties like porosity and roughness of the substrate through its parameters $k_g$ and $k_c$. Then, a very good agreement between experimental and analytical values can be found.

However, a limitation of the model is that the data set was collected experimentally and in controlled conditions. Thus, the generalization and the applicability of the model are limited to the same climatic conditions of the test and the same materials.

Consequently, to collect more data specimens of algal colonization on different experimental conditions as well as on different building materials it is necessary to enhance the predictive power of the model.

Hence, the future direction of the research could be the collection of more experimental data relative to different materials as well as to different experimental conditions.

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Author Contributions: Lorenzo Graziani and Enrico Quagliarini conceived and designed the experiments; Lorenzo Graziani performed the experiments; Lorenzo Graziani and Enrico Quagliarini analyzed and discussed, respectively, the data. All the authors wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References


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