Contact angle as a non-destructive method to determine wettability changes induced by sub-aerial biofilms on built heritage porous substrates

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Abstract

Wettability and water transport mechanisms play a relevant role in the weathering of built heritage since liquid water is directly involved in many deterioration mechanisms. Moreover, water availability can promote the colonization of microorganisms on surfaces of built heritage. While microbial colonization of stone monuments was previously considered as a significant biodeteriorative threat in heritage studies and conservation practices, recent investigations have approached it from a different perspective, shedding new light on its actual impact. Recent studies have highlighted that microbial communities, known as sub-aerial biofilms (SABs), can have a neutral or even protective role in certain situations. In the present work, a benchtop contact angle instrument was used for studying the surface wettability induced by sub-aerial biofilms (SABs) on laboratory limestone samples. Complementary information on the water interaction with the substrate in the presence of a SAB was acquired via capillary absorption tests. Field measurements of wettability and water absorption properties of biocolonized plastered wall of a historic case study, Casa a Ponente, of Palazzo Rocca in Chiavari (Genova, Italy) were also performed.

Results confirmed the potential of contact angle measurements as a non-destructive monitoring tool for the wettability of biocolonized stone substrates. The presence of SABs is associated with measurable changes in the surface wettability, resulting in near-hydrophobic conditions observed in both the lab-scale colonized samples and the case study.

Keywords: bioprotection, contact angle, hydrophobicity, non-destructive methods, sustainability, water absorption.

1. Introduction

The interactions between microorganisms and masonry surfaces are the result of the activity of complex microbial communities present on the surface as sub-aerial biofilms (SABs). Traditionally, these interactions have been considered detrimental to the substrates, leading to biodeterioration [1,2]. However, recent studies, primarily based on case studies and empirical evidence, have highlighted that SABs can have a neutral or even protective role in certain situations [3–10].

Furthermore, liquid water is a key driver for deterioration of built heritage surfaces exposed outdoor, being involved in numerous weathering and alteration mechanisms, including biocolonization. Water availability is indeed crucial in promoting SABs formation and development. Therefore, recent studies have focused their attention on the SABs/substrate interaction, particularly looking at changes in water transport proprieties and suggesting that the presence of a biofilm can induce near hydrophobic characteristics to the surfaces [11,12].

Contact angle measurement provides key information on surface wettability and water absorption. Its non-destructive and non-invasive nature, along with recent instrumentational development allowing for in-situ measurements, make it highly advantageous.

The aim of this study was to investigate wettability changes induced by SABs on built heritage porous substrates, both through lab-scale experiments and on a real case study. For the laboratory testing, limestone samples were used and colonized with a SAB model system, representative of naturally formed biofilm under outdoor conditions [13]. Such samples were analysed with a benchtop contact angle instrument to investigate their wettability in comparison to the non-colonized ones. Complementary information on the water absorption behaviour with the SAB/substrate system was acquired by capillary absorption tests.

For the onsite measurements, the biocolonized plastered wall of *Casa a Ponente,* of Palazzo Rocca in Chiavari (Genova, Italy) was selected. The *Casa a Ponente* dates back to the first half of the 17th century [14] with a building and conservation history poorly documented. The plastered wall under study, which faces northeast towards the botanical garden, recently underwent a conservation intervention aimed also at removing the patchy green patina caused by SABs. After a few months, the plastered wall experienced a biological recolonization, prompting the consideration of not pursuing further removal actions. It is therefore important to understand and monitor over time the biofilm behavior and its impact on the plastered surface, to support the decision of preserving it instead of removing it again.

2. Materials and methods

2.1 Laboratory limestone samples

A dual-species SABs composed of the phototroph *Synechocystis* sp. PCC 6803 and the heterotroph *E. coli* ATCC 25404 were grown according to the protocol reported by Villa et al. (2015) [13] modified as follows: the stone samples were immersed overnight in the planktonic culture composed of cyanobacteria and *E. coli* cellular suspensions in a 1:1 ratio. Then, the liquid culture was removed and the BG11 medium [15] was added and maintained at 3 mm above the bottom of the samples. After 5 days with a 16/8 day/night photoperiod, the SABs were mature and ready for the tests. The commercial limestone samples (7.5 cm x 2.3 cm x 1.0 cm) consisting of 90% of calcite and dolomite and with a porosity of 8.91% ± 0.78 [12] were used. Images captured using the digital microscope DinoLite Premiere AM7013MT were processed with the freely available software GIMP (GNU Image Manipulation Program, Berkeley, California) to calculate the extent of surface colonization.

After biocolonization, the samples were dried in the oven at 40°C for 24h before starting the tests.

Wettability was assessed through static water contact angle (WCA) measurements conducted on both biocolonized and uncolonized (control) samples. WCA measurements were determined using a benchtop instrument (Data Physics OCA 150, equipped with a Liquavista Stingray camera and a Liquavista LED light source for background illumination). After dosing a drop of distilled water on the surface, a video was recorded. Single frames were isolated from the video and analyzed with the software OpenDrop (GitHub, Inc., San Francisco, CA, USA). The contact angle of a water droplet decreased slowly to zero for biocolonized samples and very quickly (less than 5 seconds) for uncolonized samples. The WCA was then determined on the first frame (frame rate of 62 FPS) after the droplet touched the surface, assuming that at that moment, absorption is still negligible [11]. Furthermore, based on the number of frames, the time until complete absorption was determined.

Standard capillary water absorption tests were also performed, according to the UNI EN 10859:2000 [16].

2.2 Casa a Ponente of Palazzo Rocca

Two different areas were selected, named A01 and A02. Both have a colonized part and a non-colonized one and the plaster seems macroscopically different. The on-site investigation of the recolonized plastered wall was made by portable digital microscopic observation using a digital microscope DinoLite Premiere AM-7013MT. The percentage of recolonized surfaces was calculated as described in 2.1.

The Mobile Surface Analyzer (Krüss GmbH, Hamburg, Germany) was used for contact angle measurements: a droplet of distilled water was dosed onto the target surface and a 60 s video was recorded (frame rate of 10 FPS). The WCA was calculated similarly to the laboratory measurements, using the software KRÜSS Advance (Krüss GmbH, Hamburg, Germany) for each colonized area and compared with the non-colonized ones. Each measurement was repeated at three different points of the same area.

The contact sponge test was also used for the evaluation of the water absorption [17].

3. Results

The cyanobacteria *Synechocystis* sp. PCC 6803 with the heterotroph *E. coli* ATCC 25404 developed a thin green biofilm on the stone surface, with good adhesion to the substrate. The biofilms resulted quite homogeneous with limited areas that appeared more whitish or yellowish compared to the rest (Fig. 1a). The percentage of colonized surface of limestone samples is 91%±5.

In the case study, the SAB on the plastered wall exhibited a relatively uniform coverage of the binder, while being very thin or absent over the aggregates (Fig. 1b). The percentage of the colonized surface of the A01 and A02 areas were respectively 88%±3 and 78%±23.

The contact angle value of biocolonized samples at lab-scale was 102.5°±2.0, which was significantly higher compared to the control ones with a value of 25.6°±1.34. Moreover, in biocolonized samples, the absorption rate ranged between 5 min and 7 min, whereas the untreated limestones exhibited immediate absorption, taking less than 5 seconds for the drop to be absorbed.

A similar trend was observed in the in-situ investigation of vertical plastered colonized surfaces (Fig. 2). In this case, the presence of SAB significantly increased the contact angle. The measured contact angle value for the A01 area was 134.24°±2.2, while for A02 was 130.04°±3.4. In comparison, the uncolonized area exhibited contact angle

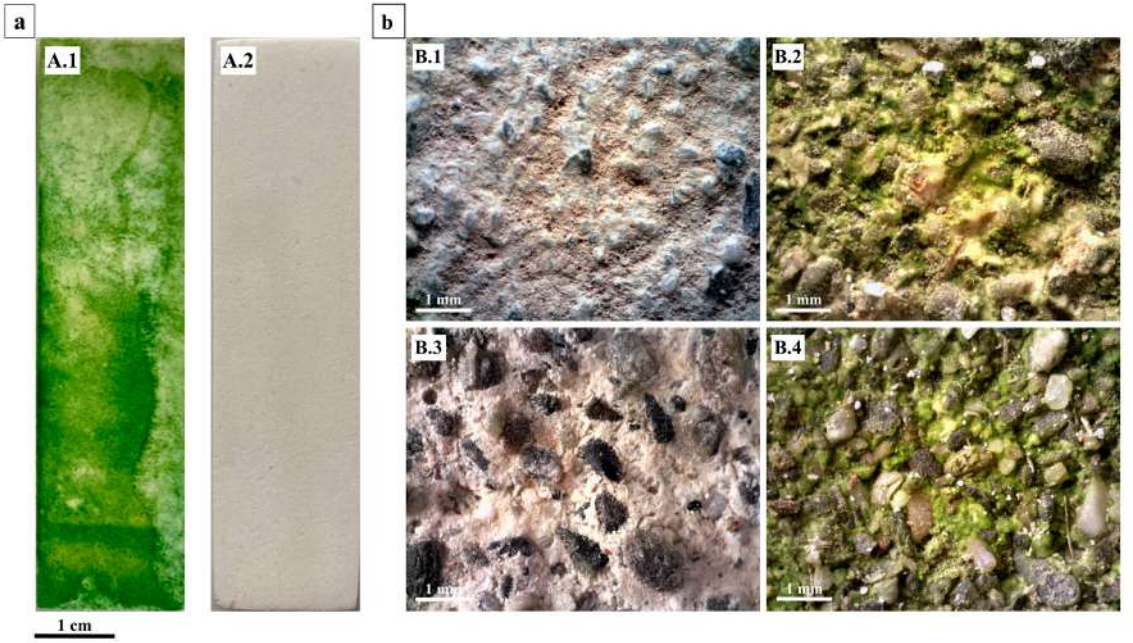


Fig. 1 (a) Macroscopic images of the limestone samples: (A.1) colonized with the dual-species biofilm and (A.2) a control sample; (b) In situ microscopy documentation of different areas of the plastered wall of *Casa a Ponente*: (B.1, B.3) without biofilm (B.1 area A01; B.3 area A02) and (B.2, B.4) biocolonized (B.2 area A01; B.4 area A02).

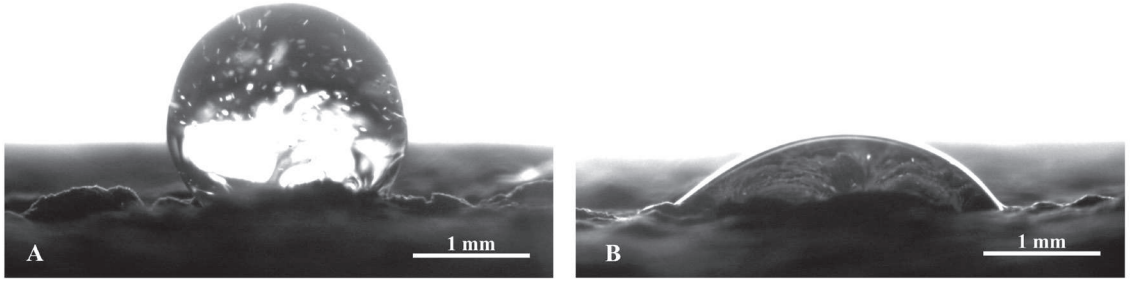


Fig. 2 First WCA. Microphotograph of (A) A01 area with green biofilm and (B) A01_NC area without biofilm.

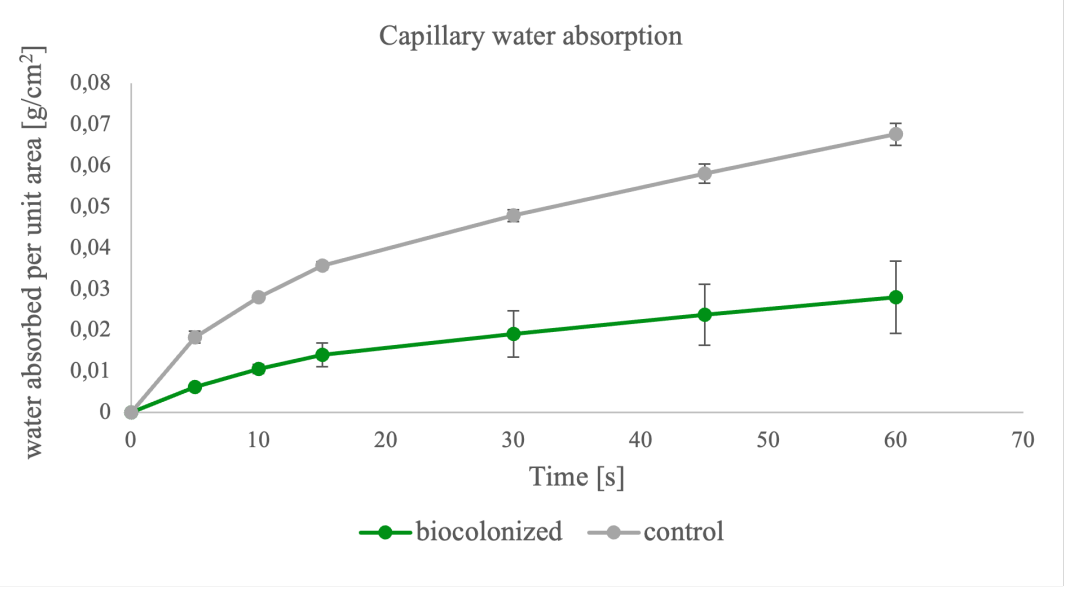
values of 45.3°±8.5 and 52.4°±8.1, respectively; the high standard deviation obtained on the areas without biofilm may be explained by considering the remarkable roughness and heterogeneity due to the inherent plaster composition. Therefore, it can be affirmed that the presence of SABs confers water-repellent characteristics to the surface [18]. Moreover, in the uncolonized areas, the water drops were absorbed by the surface within a few seconds, whereas in the biocolonized areas, the drops remained unabsorbed even after the 60-second duration of the recorded videos. In some cases, the drops volume and shape appeared almost unchanged by the end of the video.

Preliminary results of capillary absorption tests confirmed that the absorption rate in the first 60 seconds is slower in biocolonized laboratory samples than in the control ones (Graphic 1).

Similarly, in biocolonized areas, A01 and A02, of the plastered wall, the results of the contact sponge test in situ showed a lower absorbed water amount [g/cm2] compared to the uncolonized areas (respectively 14% and 42% less).

4. Conclusions

Liquid water is directly involved in many weathering mechanisms affecting masonry surfaces, and it contributes to the colonization of microorganisms. Consequently, it is fundamental to study the interaction between water and biocolonized surfaces. The contact angle measurement has been confirmed as a powerful tool for studying the surface wettability of biocolonized stones, both in laboratory and in-situ settings. The results showed that the dual-species SAB developed at lab-scale on limestone samples induced a near-hydrophobic characteristic to the surface. Similar results were also observed on the recolonized plastered wall of *Casa a Ponente* of Palazzo Rocca in Chiavari.



Graph. 1 Capillary water absorption as a function of time (first 60 s), with the control in grey and the laboratory biocolonized limestone samples in green.

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