

Environmental Adaptation of Phototrophic Biofilms on Lithic Monuments under Hot and Arid Conditions of Warangal

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Abstract:

Cyanobacteria in particular are phototrophic microorganisms that participate in biogeochemical cycles that modify the environment. They take engage in bioweathering and biomineralization processes on a variety of substrates, particularly carbonate rocks. Five phototrophic biofilms from various limestone monuments in Southern Europe were cultured in a lab setting and characterized using molecular approaches in the current work. Temperature, pH, O₂ levels, hydrodynamics, osmolarity, the presence of certain ions, nutrients, and elements derived from the biotic environment are some of the variables that influence the formation of biofilms. The pattern of activity of a particular bacterium with regard to biofilm growth is ultimately determined by the integration of various effects. Examples of how environmental factors impact biofilm formation will be provided in this chapter; the majority of these examples are drawn from research on species that have mammalian hosts. In this paper we will discuss. Environmental Adaptation of Phototrophic Biofilms on Lithic Monuments under Hot and Arid Conditions of Warangal

Keywords: Environmental Adaptation, Phototrophic Biofilms, Lithic Monuments, Arid Conditions, Microorganisms, Monuments, Micro-Habitats, Cyanobacteria, Fungus, Algae, And Heterotrophic Bacteria

Introduction:

Long-term exposure to the elements can cause monuments to degrade for a variety of reasons, including weathering from the sun, wind, rain, and pollution in the atmosphere. These pollutants can operate as catalysts of deterioration through different chemical, physical, or biological pathways, or they can react with materials and change their properties. Microbes that are different from the environment can build interface microhabitats called biofilms. Microbial communities that grow on solid mineral surfaces exposed to the atmosphere are referred to as "sub-aerial biofilms" (SAB). [1]

SAB are self-sufficient, microbial communities that are found on buildings, bare rocks in mountains and deserts, and at all latitudes where there is direct exposure to sunlight and the atmosphere. SAB may also be used as bioindicators of climatic and/or atmospheric change due to their sluggish and sensitive growth. Patchy growth, dominated by alliances of cyanobacteria, fungus, algae, and heterotrophic bacteria, is a characteristic of SAB on exposed terrestrial surfaces. The bacteria interact with physical and chemical factors to produce biofilms and serve an important role. A SAB's microbiological composition, which is influenced by the substrate's kind, exposition time, and climate, determines its color, texture, and appearance. Among the microbial consortia, phototrophs, lichens, and mosses can grow in mild, humid environments. The predominant microclimatic variables of temperature, humidity, and light regime have been identified as the primary determinants of microorganism colonization of building surfaces. [2]

The orientation of the building affects these parameters. The first organisms to colonize building surfaces were thought to be phototrophs like algae and cyanobacteria, which prepared the inert surfaces for the growth of heterotrophic species like fungi. They frequently grow on cornices, in gaps and cracks, or on buildings in humid environments. Depending on the traits of the species, the biofilms that phototrophs generate can range

in color from rose to grey and brown, and from greenish-yellow to dark green and black. Depending on the physiological cell state and the surrounding circumstances, these patinas can alter in shape and color. A damaging effect is not always implied by the presence of phototrophic bacteria on stone surfaces. However, the presence of algae on building stones can be seen as biodeteriogenic due to the aesthetic harm they create, since biodeterioration is defined as any undesired change in a material's qualities brought on by the essential activities of microorganisms. Soiling or functional degradation are terms used to describe this kind of biodeterioration mechanism. Numerous writers have discovered that the degree and type of soiling and biofilm formation are more influenced by local environmental factors than by substrate. Some authors have deemed the aggressive behavior of algae and cyanobacteria in respect to the substrate on which they grow to be inconsequential. The degradation of construction materials is facilitated by these biofilms. There are references in the literature that indicate direct deterioration mechanisms, in addition to the unsightly aspect that is apparent in the majority of reports on algae on old buildings. According to reports, epilithic cyanobacteria may actually contribute to the weathering of rock surfaces in the natural world by influencing the carbonate dissolution system in different ways. When the colonial substrata are part of cultural properties, these issues take on additional economic and social aspects.

Many monuments and historic structures have been built using sedimentary rocks, particularly limestones. This cultural legacy is particularly vulnerable to cyanobacteria and algae, which can induce biodeterioration due to a variety of microbial communities. The presence of algae and cyanobacteria on sedimentary stone objects from cultural heritage has already been evaluated in numerous researches. Because of their consequences for the biodeterioration of stone monuments, the intricate interactions between lithobionts and mineral substrates are currently of interest. [3]

The combination of physical, chemical, and biological elements causes stone monuments to weather. This process involves living microorganisms, including bacteria, cyanobacteria, fungus, and lichens, all of which are important. Lichens, microalgae, and cyanobacteria are pioneer organisms that provide nutrients to other species, thereby moderating harsh habitats. Extreme desiccation regimes are not a problem for them. Microorganisms use the endolithic lifestyle as a means of surviving harsh conditions such high sun radiation, desiccation, temperature variations, and oligotrophic conditions in the hostile environment of stone. There are four types of endolithic microorganisms: *cryptoendolithic*, *chasmoendolithic*, *euendolithic*, and *autoendolithic*. Cyanobacteria predominate in endolithic populations, which are among the least diversified ecological groups. It has been estimated that 5–6% of all cyanobacterial biomass is made up of endolithic cyanobacteria. The endolithic way of life is crucial for studying the bioweathering of historical monuments and for comprehending the relationships between microbes and minerals.

Phototrophic Biofilm Formation

The development of cyanobacteria, which are photoautotrophs and the main colonizers of light-exposed lithic habitats or substrates, contributes a substantial amount of organic matter to the stone substrates, which aids and encourages the growth of heterotrophic or chemoorganotrophic microbes such as bacteria and fungi. As a result, exposed surfaces of rocks, monuments, and buildings develop laminated subaerial biofilms (SAB). EPS holds the microbial populations of biofilms together and enables them to adhere to underlying surfaces. Light-driven and surface-attached microbial communities known as phototrophic biofilms are distinguished by the clear presence of photosynthetic organisms (such as cyanobacteria, microalgae, and diatoms), which generate and supply organic substrates and oxygen to heterotrophic microbial components. Microbial mats or phototrophic mats are common names for thick, multilayered phototrophic biofilms.

The biofilms that form on the lithic surfaces have the ability to trap pollutants, dusts, and other environmental particulates. In cultural heritage studies, crusts and patinas are terms used to describe epilithic cyanobacterial assemblages or biofilms. The development of crusts or biofilms causes the stone surface to retain moisture for longer periods of time, making it more vulnerable to additional colonization. [4]

Review of Literature:

Studying artificial phototrophic biofilms in a lab setting may improve our comprehension of the relative effects of physical and chemical changes in the substrate, while real biofilm communities are frequently challenging to research in situ. Prieto and Silva (2005), Guillitte and Dreesen (1995), Miller et al. (2006), Ortega-Calvo et al. (1991), and other researchers have created laboratory-based stone colonizations. The majority of these trials examined each member of the community independently, which made it difficult to comprehend the intricate processes of stone biodeterioration brought on by phototrophic biofilms. The knowledge of the microbiological processes underlying stone cultural heritage assets will be improved by laboratory research employing stable populations and various species. [5]

Numerous nations in tropical, subtropical, and temperate regions of the world have reported the presence of lithobiontic cyanobacteria linked to the biodeterioration of a broad variety of lithic (stone-made) monuments, architectural structures, and artifacts, including cathedrals, chapels, churches, monasteries, mosques, temples, palaces, pyramids, historical buildings, statues, tombs, and towers. They make up a significant or primarily large portion of epilithic biofilms. The lithobiontic cyanobacteria most widespread and commonly reported belong to both coccoid (unicellular and colonial) and filamentous forms, and include the genera *Synechococcus*, *Synechocystis*, *Chroococcus*, *Gloeocapsa*, *Gloeotheca*, *Chroococcidiopsis*, *Xenococcus*, *Myxosarcina*, *Phormidium*, *Lyngbya*, *Leptolyngbya*, *Calothrix*, *Plectonema*, *Pleurocapsa*, *Chlorogloeopsis*, *Nostoc*, *Microcoleus*, *cytonema*, *Tolypothrix*, *Hapalosiphon* (Macedo et al., 2009; Ortega-Calvo et al., 1995; Ortega-Morales, 2006; Lewin, 2006; Keshari and Adhikary, 2013; Tripathi et al., 1997; Gaylarde et al., 2012). [6]

In the chemical or geochemical process, the mineral lattice is weakened by the chemical compounds or metabolic products that organisms make and exude. Longer retention of the water absorbed by biofilms may promote aqueous chemical processes that cause stone weathering (Gorbushina, 2007). By having chelating and solubilizing effects on rock minerals, the synthesis of EPS, siderophores or other chelating agents, and acid or alkaline secretion are all linked to the bioweathering of natural and artificial stones. EPS can remove cationic mineral components from lithic substrates because of its anionic character. [7]

The challenge for microbiologists working in the field of cultural heritage (CH) is to comprehend the physiology and activity of biofilms (subaerial biofilms, or SABs) that inhabit outdoor stone heritage, as well as their intricate interactions with the atmosphere and mineral substrate at various temporal and spatial scales (Gorbushina, 2007; Pinna, 2014). [8]

Field research is the primary method utilized to investigate such complexity (Polo et al., 2012; Villa et al., 2015). Despite the fact that field experiments are clearly essential for comprehending the connection between SABs and ecological characteristics, a variety of obstacles hinder their planning and implementation. According to Owens et al. (2014), these difficulties include the need to preserve spatial integrity, the inaccessibility of repeated sampling, the limited and minuscule sample size, and the intricacy of the sample structure. Additionally, the temporal resolution of the experiments limits the results of many field-based studies because many processes, including succession, coevolution, invasion, and climate change, that may be significant in structuring SAB communities and activity take place over longer time scales than the average research grant, which limits our understanding of the phenomena being studied. [9]

Objectives:

- Efficiency, durability, and environmental impact of modern conservation techniques are evaluated.
- To Explain A model for biofilm development
- Advances in monument's preservation are explored.
- The microbial colonization of stone monuments is reviewed.

Research Methodology:

The physiological complexity and ecological significance of subaerial biofilms (SABs) on lithic surfaces have been clarified by recent scientific studies. Mechanistic methods for examining the spatiotemporal patterns of interactions between the biofilm, the stone, and the atmosphere are extremely important in the field of sustainable cultural heritage (CH) preservation. However, the inaccessibility of materials, the intricacy of the ecosystem being studied, and the temporal resolution of the studies have made it challenging to investigate these relationships through field experiments. In order to get over these restrictions, we set out to create a unified approach for the environmental adaptation of phototrophic biofilms on lithic monuments in Warangal's hot and dry climate.

Result and Discussion:

Significant progress has been made in the last ten years in our understanding of the physiological and genetic underpinnings of biofilm development. Planktonic and sessile cells, as well as even distinct stages of biofilm growth, exhibit strikingly diverse patterns of gene expression. However, in a few model organisms, the genetic and environmental variables that facilitate the shift from planktonic to sessile communities are still largely unknown. It is evident that distinct bacterial species and even strains can react to their surroundings in different ways. Which environmental factors make different bacterial species more likely to form a particular biofilm? How do environmental factors affect the regulation of the molecular genetic, biochemical, and structural components that mediate the formation of biofilms? In light of the biochemistry and molecular genetics of the biofilm development cycle (Fig. 1), the subsequent sections outline some of the environmental factors that affect biofilm formation. [10]

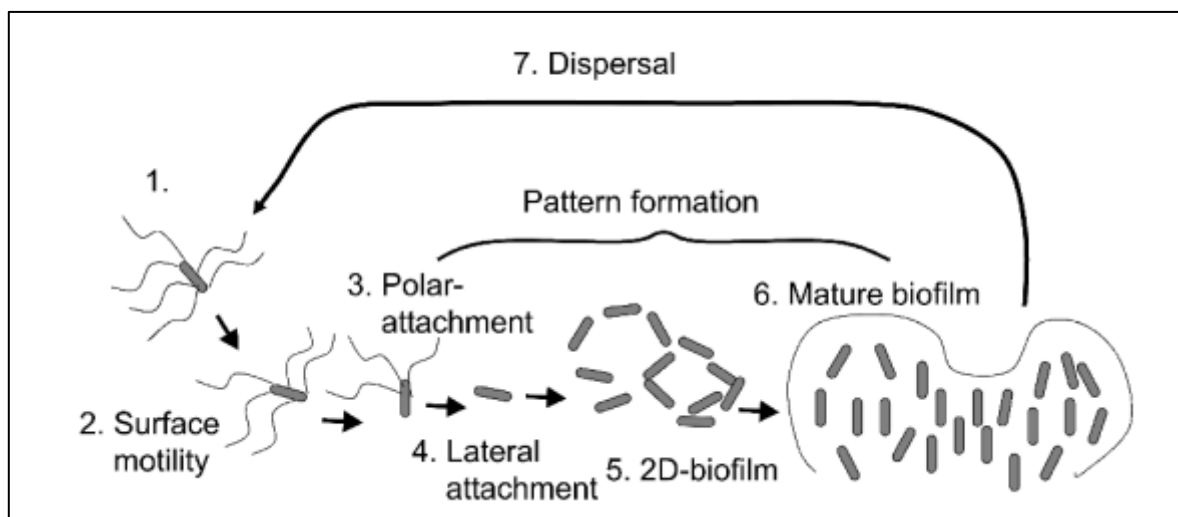


Figure 1: A model for biofilm development. (Source: ResearchGate)

In order to approach and swim on a surface, planktonic cells require motility. Cells may become reversibly attached when a pole interacts with the substratum, enabling environmental sampling prior to adopting a sessile lifestyle. After that, cells use adhesins like PGA or LapA to become laterally connected to the surface. Cell attachment starts to form a two-dimensional biofilm during this period, and in *E. coli*, the cellular dispersion shows clear periodicity. As more cells are added to the biofilm's structure, it gets thicker. More securely linked cells inside an extracellular matrix are the result of the production of extracellular polysaccharides and other chemicals. The synthesis of surfactant and the discharge of associated cells can alter the biofilm's architecture. The developmental cycle may be completed when cells are freed from the matrix and revert to a planktonic state in response to physiological or environmental cues. [11]

The current study relates rock bio-weathering and carbonate dissolution-precipitation dynamics in the semiarid Pasargadae region to cyanobacterial activity. These results also demonstrate how important water availability is for calcium carbonate weathering caused by biofilms. To elucidate interactions at the microbial community level, more investigation is required.

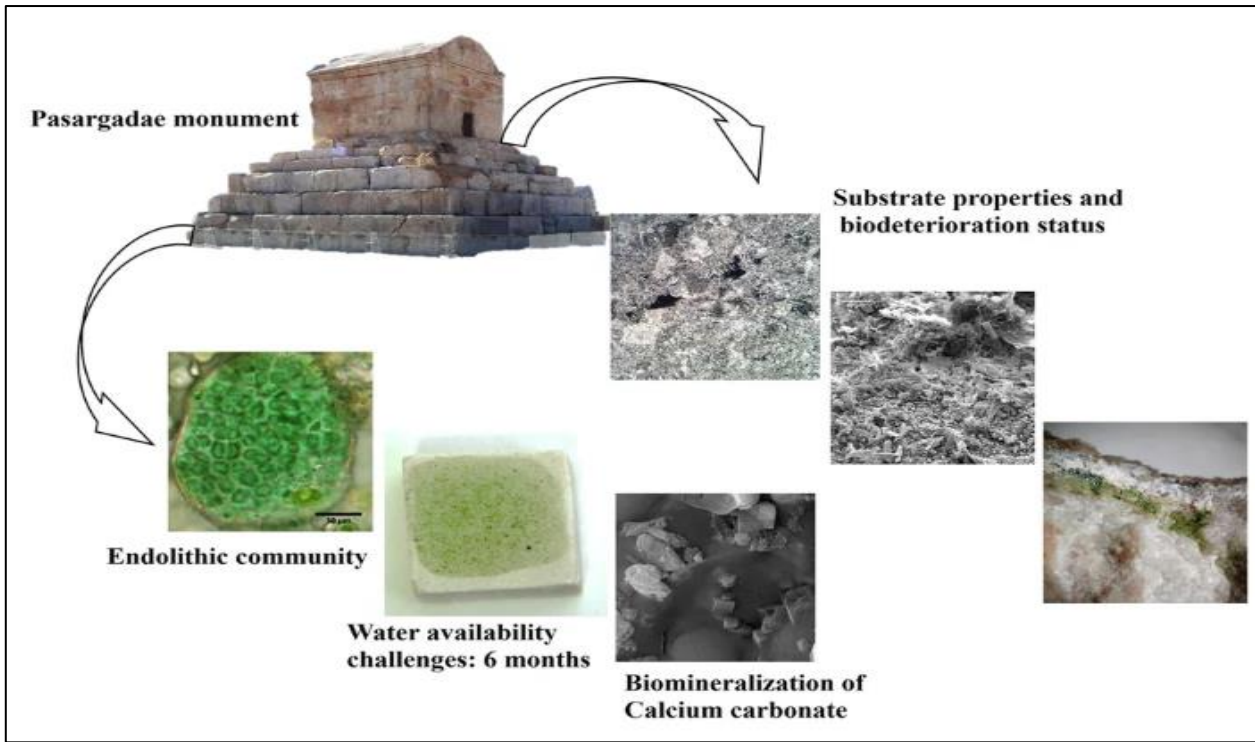


Figure 2: Wathering caused by biofilms (Source: by Google)

Limestone Characteristics

Pasargadae stone samples were found to include calcite as the primary compound, as well as various amounts of dolomite and trace amounts of quartz, hematite, alumina, and bornite, according to the results of XRD and SEM-EDX (Fig. 3B). The stone sample had uneven porosity, diagenesis, and a largely homogeneous texture (Fig. 3C, E). Thin slices of Pasargadae limestone are examined using plane-polarized light (PPL) and cross-polarized light (XPL) microscopy in Figures 3D and E, respectively. The presence of iron-containing compounds is shown by the dark hue surrounding the pores (Fig. 3D, E). Additionally, a brachiopod spine with a pseudo-uniaxial cross and polar cross was seen in the transverse section (Fig. 3D, E). [12]



Figure 3: Study area and bedrock properties. (Source: <https://www.nature.com/>)

A Pasargadae Monuments, **B** XRD analysis. The microscopic properties of the thin Pasargadae limestone piece under both polarized (**C**, **E**) and natural (**D**) light. Under polarized light, a fairly uniform texture of limestone is shown in (**C**), where the texture is crystallized, indicating diagenesis (black arrow). The calcite minerals are depicted in the thin section pictures in (**D**, **E**). The thin layer of iron-containing compounds is indicated by the black arrows. The transversal portion of a fossilized brachiopod spine is represented by the black circles. [13-14]

Conclusion:

Depending on the environment, the biofilms' ambiguous function on stone monuments might alternate between bioprotection and biodeterioration. Depending on the conditions in which the biofilms formed, the variety of algal species displayed differences. In this study, biopitting and grooves resulting from algae attacking the substrate (brick) are visible. Endolithic algae create phototrophic biofilms that release acid metabolites that aid in the geochemical processes. In addition to natural and man-made pollutants, climatic conditions like temperature and humidity can significantly intensify this kind of biodeterioration. Pollutants from the atmosphere would play a significant part in the structural materials. The Cathedral is situated in a mixed urban setting that has forceful industrial features.

REFERENCES:

1. Bjelland, T. et al. Microbial metacommunities in the lichen–rock habitat. *Environ. Microbiol. Rep.* **3**, 434–442 (2011).
2. Cámara, B. et al. Biodeterioration of marble in an underwater environment. *Sci. Total Environ.* **609**, 109–122 (2017).
3. Warscheid T, Krumbein WE (1996) General Aspects and selected Cases. In Microbially induced corrosion of materials, Heitz, Sand, Flemming Eds, Springer-Verlang, 273-295.
4. Gorbushina AA1 (2007) Life on the rocks. *Environ Microbiol* 9: 1613-1631.
5. Ortega-Calvo, J. J., X. Arino, M. Hernandez-Marine and C.Saiz-Jimenez (1991). Factors that influence how phototrophic microorganisms weather and colonize monuments. *Sci. Total Environ.*,167: 329–341.
6. Macedo, M.F., A.Z. Miller, A. Dionisio and C.SaizJimenez (2009). Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview *Microbiology*,155: 3476–3490.
7. Gorbushina, A.A. (2007). Life on the rocks. *Environ. Microbiol.*, 9(7): 1613-1631.
8. Gorbushina A. A. (2007). Life on the rocks. *Environ. Microbiol.* 9 1613–1631. 10.1111/j.1462-2920.2007.01301.x [DOI] [PubMed] [Google Scholar]
9. Polo A., Gulotta D., Santo N., Di Benedetto C., Fascio U., Toniolo L., et al. (2012). Importance of subaerial biofilms and airborne microflora in deterioration of stonework: a molecular study. *Biofouling* 28 1093–1106. 10.1080/08927014.2012.729580
10. Gómez S de Saravia, Fontana M, Guiamet P (2010) Estudio de biofilms fototróficos mediante microscopía óptica y microscopía electrónica de barrido. *Rev Argent Microbiol* 42: 315.
11. Guiamet P, Rosato V, Gómez de Saravia S, García AM, Moreno D (2012) Biofouling of crypts of historical and architectural interest at La Plata Cemetery (Argentina). *J Cult Herit* 13:339-344.
12. Cappitelli F., Salvadori O., Albanese D., Villa F., Sorlini C. (2012). Cyanobacteria cause black staining of the National Museum of the American Indian Building (Washington, D.C., USA). *Biofouling* 28 257–266. 10.1080/08927014.2012.671304
13. Decho A. W., Visscher P. T., Reid R. P. (2005). Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaios* 219 71–86. [Google Scholar]
14. De Philippis R., Sili C., Paperi R., Vincenzini M. (2001). Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *J. Appl. Phycol.* 13 293–299. 10.1023/A:1017590425924