



Influence of the properties of granitic rocks on their bioreceptivity to subaerial phototrophic biofilms



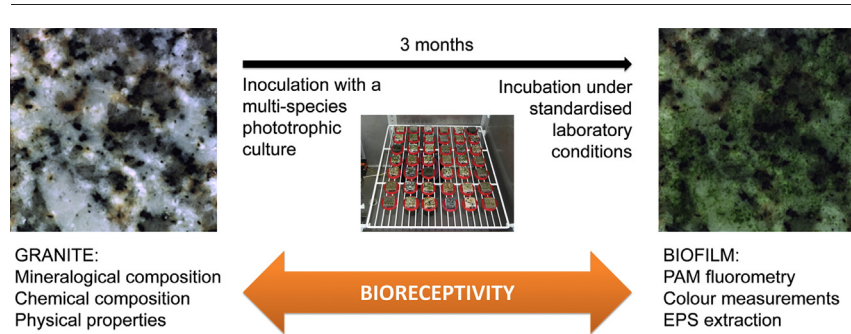
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HIGHLIGHTS

- Relationships between granite properties and biofilm growth were analysed.
- Bioreceptivity is influenced by physical properties rather than chemical composition.
- High open porosity, capillary water content and roughness promote colonisation.
- EPS matrix is produced at the initial stage regardless of subsequent growth.

GRAPHICAL ABSTRACT



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ABSTRACT

As any stone substrate is susceptible to biological colonisation, the choice of lithotype used for construction is a key strategy for preventing biodeterioration. For this purpose, a comprehensive evaluation of the primary bioreceptivity to phototrophic biofilms of eleven varieties of granitic rocks, commonly used as building material, was carried out. Blocks were inoculated with a multi-species phototrophic culture and subjected to standardised growth conditions for three months. Biofilm formation was assessed by chlorophyll (chl) fluorescence, colour measurements and extracellular polymeric substances (EPS) quantification. Relationships between the biofilm growth indicators and the properties of the different rocks studied were then analysed. Results showed that the bioreceptivity of the granites is more strongly affected by the physical characteristics of the stones than by their chemical and mineralogical properties, possibly because of the similar composition of the rocks studied. Growth of phototrophic biofilms was enhanced by high open porosity, capillary water content and surface roughness, and the bioreceptivity of weathered granites was higher than that of sound granites. The results obtained can therefore help in the selection of appropriate lithotypes for building purposes. The amounts of EPS produced by subaerial biofilms primarily depended on the requirements and/or characteristics of the biofilm-forming microorganisms, rather than on the bioreceptivity of the substratum, and microorganisms produce the amounts of EPS required at the initial stage of establishment on the stone surface, independently of the subsequent biomass development. These findings are especially important from the point of view of biodeterioration, in which the EPS matrix plays a central role.

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1. Introduction

Biological colonisation of rocks by subaerial biofilms, i.e. complex microbial communities embedded in a matrix formed by EPS, can lead to weathering, precipitation of minerals and/or protection of surfaces

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from erosion. While rock weathering in the natural environment is unquestionably essential for life on Earth (Viles, 2012), biodeterioration of stone buildings may result in high conservation and repairation costs (Prieto and Sanmartín, 2016; Villa et al., 2016; Warscheid and Braams, 2000). The extent to which a stone surface is biologically colonised depends on environmental factors and also on the intrinsic properties of the material. The choice of lithotype used for construction should therefore take into account the susceptibility of the stone to being colonised, i.e. its bioreceptivity (Guillitte, 1995). In this context, minimization of the colonisation undergone by stone structures should be considered a key strategy for preventing biodeterioration. As any stone substrate is susceptible to biological colonisation, bioreceptivity studies may help to choose the lithotype less susceptible, even if a broader preventive conservation plan is needed due to environmental conditions very favourable for biofilm growth. On the other hand, microbial colonisation of buildings is sometimes considered to be aesthetically desirable, to provide protection against some types of weathering (Bartoli et al., 2014; Viles and Cutler, 2012) and to be beneficial to the environment (Manso et al., 2015; Pérez et al., 2014). The choice of the lithotype may therefore also focus on enhancing the susceptibility to colonisation.

Several studies have been carried out to investigate the primary bioreceptivity of stone materials under laboratory conditions. Most of these experiments used individual species of microorganisms (e.g. De Muynck et al., 2009; Shirakawa et al., 2003; Tiano et al., 1995) or artificially mixed cultures (e.g. Giannantonio et al., 2009; Guillite and Dreesen, 1995; Marques et al., 2015), usually comprising cyanobacteria, green algae and/or fungi, to induce biofilm formation. As most types of colonisation occur as part of a synergistic process, colonisation by a single type of organism may either become impossible or completely atypical. The bioreceptivity of materials should thus be determined with species belonging to the major biological groups that colonise the material under study (Guillitte, 1995). Miller et al. (2009, 2010a, 2010b) used a multi-species phototrophic culture derived from a natural biofilm to inoculate various limestones. These authors highlighted the benefits of the use of this type of culture, which resembles a complex environmental community and thus enables better assessment of the bioreceptivity. Prieto et al. (2005, 2006) also produced a multi-species liquid culture composed of organisms adapted to the conditions of quartz-rich substrata, mainly cyanobacteria, bacteria and bryophytes. These researchers then used the culture as an inoculum to induce biofilm formation on open rock faces in a quartz quarry, with the aim of reducing the visual impact generated by quartz mining and thus demonstrating the value of these types of culture for field applications.

The types of rock most commonly studied regarding their bioreceptivity are carbonate rocks (e.g. Favero-Longo et al., 2009; Guillite and Dreesen, 1995; Miller et al., 2009, 2010b; Morillas et al., 2015; Tomaselli et al., 2000). The potential colonisation of artificial stone material (e.g. bricks and concrete) is also quite well documented (e.g. De Muynck et al., 2009; D'Orazio et al., 2014; Giannantonio et al., 2009; Guillite and Dreesen, 1995; Shirakawa et al., 2003). Some researchers have even designed cementitious materials with physico-chemical properties specifically aimed at enhancing the bioreceptivity (Manso et al., 2014a, 2014b), as the biological colonisation of facades can also be considered beneficial in modern constructions, from an ecological point of view. However, the bioreceptivity of granitic stones has been less well investigated (Marques et al., 2015; Miller et al., 2006; Prieto and Silva, 2005; Prieto et al., 2014; Tiano et al., 1995). In general, the potential bioreceptivity of stone materials has not been clearly correlated with the physical or chemical characteristics, although some properties such as the surface roughness, open porosity and mineralogical nature of the stones are considered key factors (Guillite and Dreesen, 1995; Miller et al., 2006, 2009; Prieto and Silva, 2005; Shirakawa et al., 2003; Silva et al., 1997; Wiktor et al., 2009). The aforementioned studies have provided very valuable information concerning some of the stone characteristics influencing the susceptibility of granite to colonisation and have established the first comparisons with other

lithotypes; however, further research is clearly needed to overcome many remaining uncertainties.

In a previous work, Vázquez-Nion et al. (2016a) produced stable multi-species phototrophic cultures derived from natural subaerial biofilms grown on historical granite buildings in Santiago de Compostela (NW Spain). These cultures were taxonomically characterized and proved to be composed by common pioneer colonisers of building stone surfaces, including granite. Vázquez-Nion et al. (2017) also developed a standardised laboratory protocol in which multi-species phototrophic cultures were used to induce environmental-like colonisation of granitic stone. The potential use of these cultures as inocula in experiments aimed at studying the bioreceptivity of granitic rocks was also evaluated. One of these cultures, comprising several taxa including Bryophyta, Charophyta, Chlorophyta and Cyanobacteria, was particularly suitable for this purpose due to its microbial richness, rapid adaptability to the substratum and high capacity for colonisation.

The aim of the present study was to assess the influence of the physical and chemical properties of several types of granite, commonly used as building material and/or ornamental stone, on their primary bioreceptivity to phototrophic biofilms. For this purpose, rock samples were physically, chemically and mineralogically characterized. By using the aforementioned inoculum and standardised laboratory protocol, biofilms were grown on granite blocks and the extent of colonisation achieved was assessed by chlorophyll fluorescence, colour measurements and EPS extraction. The data obtained were used to study the relationships between the properties of the granites and their susceptibility to biological colonisation.

2. Materials and methods

2.1. Lithotypes studied

Fresh cut quarry samples of eleven varieties of rocks, commonly used as construction materials in Spain and commercially available under the denomination 'granite', were selected for this study (Table 1). All the stone samples were subjected to the same surface finish (honed) in order to establish comparisons between the types of rock independently from their surface roughness. One of the lithotypes (granite SIN) was additionally subjected to different surface finishes (polished, honed, sawn and sanded) in order to assess the influence of the roughness on the bioreceptivity independently from the inherent physical and chemical properties of the stone. Different surface finishes were studied for only one of the granites in order to keep a number of samples affordable for laboratory-induced biofilm formation, and a similar effect of the surface roughness on the bioreceptivity observed for granite SIN will be therefore assumed for the other lithotypes.

2.2. Characterization of lithotypes

Rocks were petrographically characterized by the observation of thin sections under polarized light optical microscopy, with special attention to the mineralogy, texture and grain size, as well as to possible mineral weathering patterns. The mineral composition (modal analysis) of each sample was carried out by the point counting method, using a quadrilateral mesh with apertures of 0.5–1.0 mm (ca. 750–1300 points per thin section). The grain size was determined by measuring the area of the crystal sections for the major minerals.

The chemical composition of the different lithotypes was spectroscopically determined after complete acid digestion. For each sample, 4 mL of HF 48%, 4 mL of HNO₃ 60% and 2 mL of H₂SO₄ 96% were added to 0.25 g of rock powder (<50 µm) in a Teflon vessel and digested in a microwave oven (CEM Mars Xpress) by applying the following heating program: 180 °C, ramp 10 min, hold 15 min. After that, 30 mL of saturated boric acid (60 g L⁻¹) were added to the vessel and digested at 160 °C, ramp 10 min, hold 10 min (modified from Silva et al., 1999). The major elements in the resulting extracts were then analysed by

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