Applied Surface Science 399 (2017) 758–768

Contents lists available at ScienceDirect

Applied Surface Science

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

Full length article

Influence of wavelength on the laser removal of lichens colonizing heritage stone

M. Sanz[a,*], M. Oujja[a], C. Ascaso[b], S. Pérez-Ortega[c], V. Souza-Egipsy[d], R. Fort[e], A. de los Rios[b], J. Wierzchos[b], M.V. Cañamares[d], M. Castillejo[a]

[a] Instituto de Química Física Rocasolano (IQFR-CSIC), Serrano 119, 28006, Madrid, Spain
[b] Museo Nacional de Ciencias Naturales (MNCN-CSIC), José Gutiérrez Abascal 2, 28006, Madrid, Spain
[c] Real Jardín Botánico (RJB-CSIC), Plaza de Murillo 2, 28014, Madrid, Spain
[d] Instituto de Estructura de la Materia (IEM-CSIC), Serrano 121, 28006, Madrid, Spain
[e] Instituto de Geociencias (IGEO-CSIC, UCM), José Antonio Nováis 12, 28040, Madrid, Spain

ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form
28 November 2016
Accepted 5 December 2016
Available online 6 December 2016

Keywords:
Laser removal
Lichens
Biodeteriogen layer
Stone
Conservation

ABSTRACT

Laser irradiation of lichen thalli on heritage stones serves for the control of epilithic and endolithic biological colonizations. In this work we investigate rock samples from two quarries traditionally used as source of monumental stone, sandstone from Valonsadero (Soria, Spain) and granite from Alpedrete (Madrid, Spain), in order to find conditions for efficient laser removal of lichen thalli that ensure preservation of the lithic substrate. The samples presented superficial areas colonized by different types of crustose lichens, i.e. *Candelariella vitellina, Aspicilia viridescens, Rhizocarpon disporum* and *Protoparmeliopsis muralis* in Valonsadero samples and *P.* cf. *bolcana* and *A.* cf. *contorta* in Alpedrete samples. A comparative laser cleaning study was carried out on the mentioned samples with ns Q-switched Nd:YAG laser pulses of 1064 nm (fundamental radiation), 355 nm (3rd harmonic) and 266 nm (4th harmonic) and sequences of IR-UV pulses. A number of techniques such as UV-Vis absorption spectroscopy, stereomicroscopy, scanning electron microscopy (SEM) at low vacuum, SEM with backscattered electron imaging (SEM-BSE), electron dispersive spectroscopy (EDS) and FT-Raman spectroscopy were employed to determine the best laser irradiation conditions and to detect possible structural, morphological and chemical changes on the irradiated surfaces.

The results show that the laser treatment does not lead to the complete removal of the studied lichen thalli, although clearly induces substantial damage, in the form of loss of the lichen upper cortex and damage to the algal layer. In the medium term these alterations could result in the destruction of the lichen thalli, thus providing a degree of control of the biodeterioration processes of the lithic substrate and reducing the chances of subsequent lichen recolonization.

1. Introduction

Free living microorganisms as fungi, algae, cyanobacteria and non-photosynthetic bacteria, as well as lichens, symbiotic associations between fungi and algae/cyanobacteria, represent the main biodeterioration agents on stone substrates [1–3]. Highlighting recent studies on photoautotrophic microbiota, it has been found that algae are responsible of a certain degree of aesthetic damage, leading to the green staining of surfaces. In contrast, cyanobacteria can also induce endolithic damage, altering crystalline surfaces of the stone [4,5]. Generally, the internal regions of the lithic substrate are also subjected to colonization by endolithic fungi that lead to significant physical and chemical effects and at times result in considerable substrate damage [6,7]. On the other hand, lichens can affect the surface of the stone and can also penetrate deep into the substrate, promoting physical and chemical changes [8]. As epilithic and endolithic colonizers play an important role in biodeterioration of stone, microscopic techniques are necessary to evaluate their action. In situ microscopy, which consist of simultaneously applying several microscopy techniques without separating the biological component from its habitat [9], allows characterization of the biofilms involved in biodeterioration and reveals the biogeophysical and biogeochemical impact of biodeteriogen agents.

The effective removal of microbial colonization is necessary for the preservation of stone heritage. Mechanical treatments often fail to protect the substrate integrity and the application of biocides, apart from involving environmental toxicity issues, is not always completely effective to eliminate biodeteriogens [1,2]. The main

* Corresponding author.
E-mail address: mikel.sanz@iqfr.csic.es (M. Sanz).

http://dx.doi.org/10.1016/j.apsusc.2016.12.032
0169-4332/

problem detected during biocide treatment of lichens encrusted on stone heritage is that considerable portions of lichen thalli remain attached to the substrate, even after several weeks of application of highly concentrated biocides, usually applied with bristle brushes or in the form of poultices [1,2,10]. In previous treatments of lichen encrustations, a mixture of dead and living microorganisms was found after biocide application. These included living fungal forms in stone cracks and debris of dead, free-living and lichenized fungi [11]. Other treatments were effective for cyanobacteria, but with unequal effects in lichen and endolithic fungi [11,12].

Laser cleaning constitutes a promising alternative to more conventional cleaning techniques for certain applications. In the field of cultural heritage, it is a well established technique, allowing fine and selective removal of superficial deposits and encrustations [10,13–25]. Laser irradiation can be used for antifouling purposes, causing mortality to biofilm forming microorganisms, like marine bacteria and microalgae [26,27]. When applied to the treatment of lichen encrustations on stone, and although laser cleaning fails to completely remove lichen thalli, superficial lichen debris can be brushed away without affecting the substrate surface [10,28,29]. When applied to microbial colonization on stone, the laser cleaning approach requires a physical parametrization, associated with a detailed petrographic and mineralogical diagnosis of the effects induced on the substrates, allowing the determination of the irradiation thresholds for damage phenomena and the understating of the nature of the effects on biodeteriogens and stones [28,30–32]. Depending on the microorganism type and species involved, different complex interactions with the rock minerals are observable [6,7].

Some laser cleaning studies have focused on the removal from stone of biodeteriogen agents such as epilithic lichen and fungi [10,28–34]. It has been shown that Er:YAG laser irradiation (at 2.94 μm) produces cellular destruction in the lichen *Diploschistes scruposus* due to the laser wavelength coincidence with the OH absorption band of either intrinsic or extrinsic water [28]. A rapid heat transfer to lichen thalli could take place when the laser wavelength is in resonance with light absorption bands of biodeterioration layers. For the lichen species *Verrucaria nigrescens*, Q-switched nanosecond Nd:YAG laser irradiation causes the partial removal of lichen thalli, with clear structural and ultrastructural alterations, including the ablation of the lichen upper cortex and part of the medulla and the plasmolysis of endolithic fungal cells [32]. These effects are probably due to the high concentration of endogenous or exogenous absorbing compounds at 1064 nm. A study of the effect of pulse duration and wavelength of Nd:YAG laser in the elimination of *V. nigrescens* from Carrara marble substrates has shown the advantages of irradiation at 532 nm, mainly due to the corresponding high optical absorption of the fungal melanin compound [30,34], with the ensuing disruptive photothermal and photomechanical effects. Very recently, some of us [31] compared different Q-switched ns Nd:YAG irradiation modes based on single-wavelength irradiation, at 1064 nm and at 355 nm, and on a double wavelength irradiation consisting in sequences of a number of 1064 nm pulses followed by the same number of 355 nm pulses. We showed that this sequential IR–UV laser treatment ensures effective removal of lichen thalli and damage in the microbial cells, thus providing a method for effective elimination of crustose epilithic lichens.

In this work, samples of sandstone from Valonsadero (Soria, Spain) [35] and of granite from Alpedrete (Madrid, Spain) [36] were investigated in order to find the conditions for efficient laser removal of crustose lichens, typically colonizing these two types of lithic substrates. The selected stone types are traditionally used in heritage buildings and monuments in central Spain and the samples studied presented superficial areas colonized by different crustose lichens, i.e. *Candelariella vitellina, Aspicilia viridescens, Rhizocarpon disporum* and *Protoparmeliopsis muralis* in Valonsadero samples and *P.* cf. *bolcana* and *A.* cf. *contorta* in Alpedrete samples. These species show a wide range of anatomical structures and a diverse set of secondary compounds. UV–vis absorption spectra of the biodeteriogen layer diluted in ethanol and identification of the lichen secondary compounds by thin layer chromatography (TLC) served to determine the best laser wavelengths for irradiation of each biodeteriogen species. A laser cleaning study was carried out on the mentioned samples with ns Q-switched Nd:YAG laser pulses of 1064 nm (fundamental radiation), 355 nm (3rd harmonic) and 266 nm (4th harmonic) at fluences just below the previously determined ablation thresholds of the bare stone samples.

A number of techniques were employed to detect morphological and chemical changes on the irradiated surfaces. Stereomicroscopy was used to describe morphological and colour changes, scanning electron microscopy (SEM) at low vacuum served to analyse the effects on the surface of the lichens, while SEM with backscattered electron imaging (SEM-BSE) and electron dispersive spectroscopy (EDS) microanalyses of the polished transversal cross sections were applied to assess effects inside the lichen layer. FT-Raman spectroscopy was employed to detect possible structural and chemical changes of stone substrates.

2. Experimental and methodology

The present study was carried out on sandstone samples consisting of quartz (60–85%), K feldspar (10–30%) and mica (2–5%) [35] and granite samples consisting of interlocking plagioclase aggregates (20–30%), quartz (30–40%), K feldspar (25–35%) and biotite (10–20%) [36].

Elemental chemical analysis of the two stone types was carried out using between 8 and 15 kg of fresh rock samples, that were dissolved in a mixture of HF and $HClO_3$ in platinum crucibles. Major elements were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Jovin-Yvon 38 plus spectrometer. FeO was determined by titration.

According to these measurements, the chemical composition (in wt%) of sandstone samples was: SiO_2 (76.0), Al_2O_3 (10.0), Fe_2O_3 (5.9), K_2O (3.6), Na_2O (0.24), CaO (0.06), MgO (0.08), TiO_2 (0.23), SO_3 (<0.03) and of granite samples: SiO_2 (69.6), Al_2O_3 (15.02), Fe_2O_3 (2.97), FeO (1.54), K_2O (3.89), Na_2O (3.32), CaO (2.45), MgO (0.96), TiO_2 (0.4), P_2O_5 (0.16), MnO (0.05). These values are the result of averaging over a high number of samples of the same stone and are affected by less than 1% error. It has to be noticed that the CaO concentration of granite is considerably higher than that of the sandstone, a factor that can influence the lichen removal, as it will be seen in the Results and Discussion section.

As mentioned, sandstone samples were colonized by the crustose lichen species *Candelariella vitellina* (yellow-orange), *Aspicilia viridescens* (greenish grey), *Rhizocarpon disporum* (grey brown) and *Protoparmeliopsis muralis* (yellowish green) and granite samples were colonized by the also crustose lichens *P.* cf. *bolcana* (yellowish green) and *A.* cf. *contorta* (greenish grey).

To determine the best laser wavelength for irradiation in each case, we employed a double approach. First, we measured the UV-Vis absorption spectra of extracts of lichen layers. To that purpose, we dissolved ca. 1 cm^2 of each lichen sample in 5 mL of pure ethanol during 16 h. The resulting suspensions were then filtered with a 0.2 μm Millipore syringe filter. Spectra were measured in the 200–1100 nm range with a double-beam spectrophotometer (Shimadzu UV-3600) using 1 cm optical path quartz cuvettes for both the sample and for the pure ethanol reference. Second, we identified lichen secondary compounds by TLC following the method of Orange et al. [37]. In brief, ca. 1 cm^2 of each lichen species was immersed in 400 μL of acetone during 60 min. The extracts were