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New protective coatings against lampenflora growing in the Pommery Champagne cellar

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ABSTRACT

Phototrophic microorganisms such as cyanobacteria and microalgae can proliferate readily in underground heritage sites where the introduction of artificial illumination equipment has significantly altered previously stable environmental conditions. The extended lampenflora biofilm growth on the bas-reliefs carved in the underground Pommery Champagne cellar in Reims (France) represents a recurring biocolonisation problem which requires periodic cleaning. The aim of this work was to limit the growth of lampenflora on chalk substrates using preventative biocidal treatments based on polyoxometalate ionic liquids (POM-ILs). Biocidal assays carried out in laboratory showed how two different colourless POM-IL coatings were more effective than commercial Preventol RI80 against two algal strains isolated from the Pommery bas reliefs, *Pseudostichococcus monallantoides* and *Chromochloris zofingiensis*. However, only one POM-IL variant was capable of sustained prevention of biofilm growth when applied to wet chalk, which replicates the more drastic natural environmental conditions of the cellar and can limit the performance of the biocidal coatings. Crucially, coating concentration studies demonstrate how POM-IL-coated slabs from previous experiments retain their biocidal activity and can prevent subsequent recolonisation following the re-inoculation of coated slabs with algae and cyanobacteria. Consequently, POM-ILs represent excellent candidates to eliminate lampenflora growth on the chalk bas-reliefs in the unique subterranean environment of the Pommery Champagne cellar.

1. Introduction

The mechanical fracturing and disintegration of natural stone substrates by lithobionts and corrosion through the metabolic processes of microorganisms represent the greatest biodeterioration threats to the conservation of stone-based cultural heritage, however, undesired biological colonisation of stone materials can also lead to unwanted aesthetic changes such as unsightly discoloration that covers art details (Warscheid and Braams, 2000; Charola et al., 2011; Dias et al., 2020; Favero-Longo and Viles, 2020). The open-air conditions of exposure of buildings and monuments induce a large influence of climatic factors on the bioweathering by changing of sunlight radiation, temperatures and rainfall which vary with respect to their geographical location and ongoing climate change. Underground cultural heritage like caves, in the natural state, generally have a weak connection with the external atmosphere (Sanchez-Moral et al., 2021) and are considered to be extreme environments for microbial growth, due to the low nutrient availability (Simon et al., 2007). In such environments,

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chemolithoautotrophic organisms can thrive on stone surfaces as a result of stable conditions, constant air and water temperature, high moisture and solute-rich groundwater (Bastian and Alabouvette, 2009). However, underground caves now represent appealing tourist attractions (Cigna and Forti, 2013) and the introduction of lighting equipment can rapidly and significantly alter previously stable environmental conditions by increasing ambient temperature and CO_2 concentration, and decreasing relative humidity (Mulec et al., 2012; Baquedano Estévez et al., 2019; Caneva et al., 2020). Furthermore, intensified human activity also brings organic matter and new strains of microorganisms from outside which gradually adapt to subterranean environments (Mulec, 2014; Marques et al., 2016). One reason for this is that increased geotourism can foster the perfect combination of microbes that typically thrive on the surface along with the need for well-lit and illuminated areas, meaning that phototrophic organisms (primarily cyanobacteria and microalgae) proliferate readily (Mulec and Kosi, 2009). As a result, the biocolonisation of paintings and artifacts by the lampenflora (Dobat, 1969) has led to an irretrievable loss of historical heritage and an extinction of original cave-dwelling organisms (Bastian et al., 2010; Albertano, 2012; Perez, 2018).

One such example of this is the extended lampenflora growth on the bas-reliefs carved in the prestigious underground Pommery Champagne cellar in Reims, located in a French region called Grand Est. Wells act as a weak source of natural sunlight, but it is the artificial lights - first with candles and then through electric illumination – that are the primary disruptors of the natural ecosystem. Several halls are decorated with monumental bas-reliefs carved in the chalk by a French sculptor, Gustave Navlet, between 1882 and 1885, and are listed as UNESCO World Heritage (Fig. 1a and b). Many tourist visits are organised every day to present Champagne cellar galleries and halls where chalk bas-reliefs are highlighted by incandescent lamps which have been replaced latterly by LED. Moreover, contemporary art works have also been exhibited in halls over the last 13 years, which have enhanced the need for artificial light. In addition, some of them are luminous which generate excess light (Fig. 1c and d). The inappropriate increase of artificial light and increase in tourism have raised the outside air-current and led to the growth of lampenflora on bas-reliefs. The progressive spread of the lampenflora has formed extensive greenish and reddish biofilms which disturb the beauty of the bas-reliefs and contributes to their deterioration (Albertano and Urzì, 1999; Borderie et al., 2014; Bruno et al., 2019; Nikolić et al., 2020). The microbial communities are removed every six months using a classic cleaning procedure involving a soft airbrush with bicarbonate, but the lampenflora biofilms rapidly recolonise bas-reliefs, which has increased the need for periodic cleaning and more extreme measures. In this respect, the bas-reliefs have had to be repaired frequently in recent years and have left visible "scars" indicating where biocolonisation once caused physical alterations to the stone carvings.

The aim of this work was to limit the growth of lampenflora on chalk substrates. Drastic solutions such as the closure of the site to tourists, similar to the case of Lascaux cave in 1963 and others since, is improbable in the Pommery Champagne cellar context because the cellar tours represent a key factor for the promotion of Champagne and provide important income. In many subterranean sites (e.g., caves, catacombs, and hypogea), the common cleaning procedure consists of curative methods with hypochlorites which rapidly degrade organic substances through oxidation. But this approach can release poisonous chlorine gas, which has a harmful consequence on the environment and, in the case of the chalk which constitutes the Pommery cellar, the release of chloride ions could also lead to a dissolution of the stone and to the reddish colouring of the bas-reliefs *via* the oxidation of Fe^{2+} present in the chalk. Hydrogen peroxide is also used but it has a strong oxidising effect and produces corrosion of the material (Mulec and Kosi, 2009). Biocides like quaternary ammonium salts are also effective on many microorganisms (e.g., fungi, algae, and lichen) (Ascaso et al., 2002; Urzì et al., 2016; Vannini et al., 2018; Li et al., 2020) as a curative treatment, but the challenge here was to provide a long-lasting biocidal effect to avoid the lampenflora growth despite a subterranean environment which meets favourable conditions to promote it.

Recently, polyoxometalate-ionic liquids (POM-ILs) have gained attention in the heritage field as protective antimicrobial coatings, due to their tailorable antimicrobial properties, offered by their structural and compositional versatility (Hallett and Welton, 2011; Zakrewsky et al., 2014). These compounds, which are salts with a melting point below 100 °C, are composed of an anionic metal-oxide cluster (polyoxometalate, POM for short) and an organic cation (typically a quaternary ammonium or phosphonium cation) and have been previously reported as effective antimicrobials against different microorganisms (Kubo et al., 2017; Misra et al., 2018). Their hydrophobicity, high antimicrobial and anticorrosion activity combined with generally colourless nature, means that they can be applied at low concentrations as transparent coatings to prevent biodeterioration in heritage objects and architecture (Franco-Castillo et al., 2021). We have demonstrated how

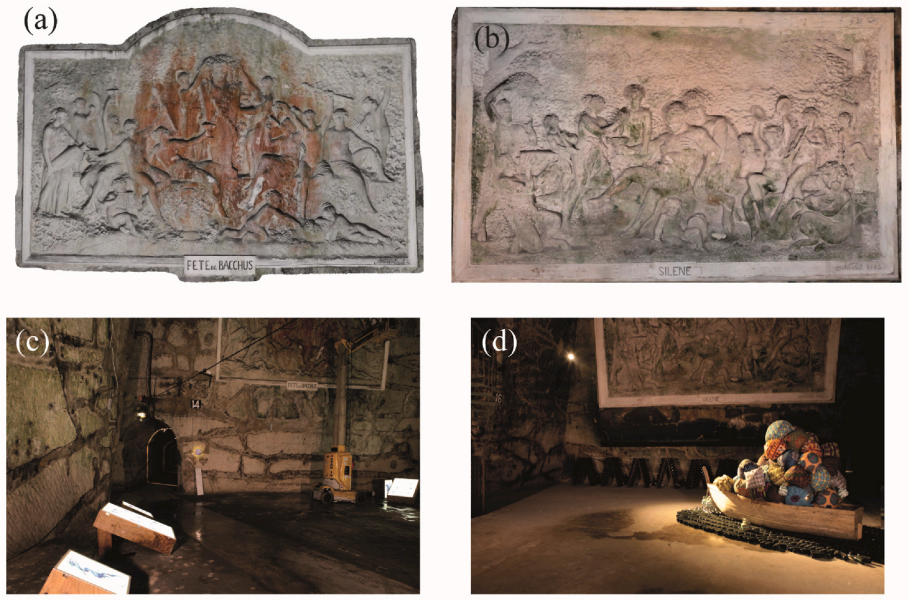

Fig. 1. "Fête de Bacchus" (5 × 8 m) (a) and "Silène" (15 × 5 m) (b) bas-reliefs carved by the French sculptor Gustave Navlet in the Pommery Champagne Cellar between 1882 and 1885. Images were built by photogrammetry from respectively 94 and 153 photos (Coolpix B700) by Agisoft Metashape v.1.7.2 software (S. Laratte). Photos of the cellar room with "Fête de Bacchus" and lighting contemporary artworks (c); cellar room with "Silène" and a contemporary artwork lighted by a spot. Formerly a ramp with 10 spots distributed under and along the bas-relief (d) (S. Laratte).

POM-ILs can prevent biofilm formation and corrosion of natural limestone of varying porosity (Misra et al., 2018). Recently, we also reported how POM-ILs can act as disinfectant agents to eliminate mould colonisation over brick surfaces (Rajkowska et al., 2020). Others have evaluated their ability to inhibit bacterial colonising of wall paintings (Li et al., 2021).

Here we explore the activity of two different POM-ILs as protective coatings against lampenflora colonisation of natural stones and compare their activity with the commercially available Preventol RI80 (PR80). The aim of this research is to evaluate the use of POM-IL1 and POM-IL2 against algae and cyanobacteria present in the bas-reliefs as well as their mode-of-application and duration of action. Both POM-ILs are based on the same Keggin-type anion, $[\alpha\text{-}SiW_{11}O_{39}]^{8-}$, and two different tetraalkylammonium ions (tetraheptylammonium and trihexyl tetradecyl ammonium, POM-IL1 and POM-IL2, respectively).

2. Materials and methods

2.1. Presentation of the Pommery cellar's stone

Pommery Champagne cellar is located in the basement of Reims city and is made of Chalk. This stone belongs to the Senonian age (89.8–66 My) of Cretaceous Period and represents 5% of the bedrock surface of West-Central Europe (Ballesteros et al., 2021). In Reims, Chalk has been quarried as a construction material from 10th to 15th centuries for the building of religious monuments. This stone was then replaced by Lutetian limestones in churches, but it is still employed for castles and private houses (Fronteau, 2000). Chalky quarries have been used age Champagne wine since the 19th century. The Pommery Champagne cellar is situated at a depth of 30 m depth and is composed of 18 km of underground galleries and halls. The temperature was respectively 17.6 °C and 18.6 °C in "Fête de Bacchus" and in "Silène" rooms in June 2020 and 92% of relative humidity in both rooms. In February 2021, the temperature was 14.1 °C and 15 °C in "Fête de Bacchus" and "Silène" rooms with the relative humidity of 95%. The high fracturing of the stone provides a wetness inside the stone and increases the relative humidity in the cellar, especially in rainy seasons like in winter.

The stone is a homogeneous coccolithic limestone with a high porosity (40.4%). The porous network was analysed by mercury (Hg) intrusion measurements and is characterised by 98.7% of micropores with a diameter less than 1 μm (most of them *ca.* 0.25 μm). The strong capillary coefficient of 352.5 g $m^{-2}.s^{-1/2}$ highlights a very good connection of pores and a high water saturation (94.4%). Therefore, these results indicate that, *a priori*, chalk is a material with a high bioreceptivity due to the fact that it can absorb and release a large amount of water (over long periods of time), making it readily available to lampenflora microorganisms colonising the cellar walls (Miller et al., 2012).

2.2. Biocides

POM-ILs 1 and 2 were synthesised according to a previously reported synthetic procedure (Misra et al., 2018). Preventol RI80 is a solution of quaternary ammonium salts as Benzalkonium chloride (alkyl-dimethylbenzylamine chloride (80%) and isopropanol (2%)) - Lanxess, Köln, Germany. Quaternary ammonium compounds are widely used as antiseptics and disinfectants in medical and industrial fields (Gilbert and Moore, 2005). In cultural heritage, those components are commonly used during cleaning procedures by professional restorers (Stupar et al., 2014; Favero-Longo et al., 2018). The efficiency of Preventol RI80 was proven in many studies as a curative treatment to eliminate a wide spectrum of microorganisms, such as fungi and bacteria (Blazquez et al., 2000; Maxim et al., 2012). It was also proven to be efficient against photosynthetic microorganisms such as algae, cyanobacteria, lichen (Ascaso et al., 2002; Vannini et al., 2018; Genova et al., 2020), complex biofilms on outdoor monuments (Coutinho et al., 2016; Sanmartín et al., 2020) and in subterranean environments (Nugari et al., 2009; Urzì et al., 2016). For our study, biocides were applied on non-colonised stone samples, as preventive treatments to avoid the settlement of the lampenflora microorganisms. For this purpose, both POM-IL1 and 2 were diluted in acetone to achieve a concentration of 100 mg/mL and 2% of Preventol RI80 was diluted in distilled water (as recommended).

2.3. Biocide coating on stone samples

Diluted solutions were applied by brush on stone slabs measuring 5 × 5 × 1 cm. The quantity of biocide applied on each stone sample was controlled by weighing to obtain similar quantity for triplicates. After 48 h of drying, slabs were weighed to calculate the effective quantity of coating remained on stone for the biocidal assays (Table S1). For assay 1 and 2, liquid coatings were applied on dry stones while for assay 3 and 4, each stone was saturated with 9 mL of distilled water before the application in order to simulate to the real application on the cellar walls, which are close to saturation. Under such conditions, the biocidal coating remained on the surface of the stone without being absorbed and penetrating the pores of the chalk. Accordingly, three different amounts of POM-ILs solutions were brushed (0.1, 0.2, and 0.4 g) and two different amounts of Preventol RI80 (0.2 and 0.4 g) since its biocidal effect was weaker, even at the highest tested concentration of 0.4 g in assay 2.

2.4. Identification of algae and cyanobacteria for biocidal assays

The sampling of biofilms was carried out from a visual selection on the coloured areas and on the non-coloured area (Fig. S1). The biofilm samples collected from both bas-reliefs of the Champagne cellar were cultured on agar plates with Hoagland medium to isolate algae and cyanobacteria species. DNeasy PowerBiofilm kit (Qiagen) was used for DNA extraction following the manufacturer's instructions. The 18S rRNA genes were amplified by PCR using eukaryote-specific primers, the forward primer NS1F (5′-GTAGTCATATGCTTGTCTC-3′) and the reverse primer NS8R (5′-TCCGCAGGTTCACCTACGGA-3′), the thermal cycling profile started by an initial denaturation at 95 °C for 5 min, then 30 cycles at 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min 30 s, followed by a final extension at 72 °C for 10 min. Only one alga species was identified with the universal primers (Table S2) and for better results, *rbc*L genes of the plastid genome were used as forward primer S1F (5′-ATGTCACCACAAACAGAGACTAAAGC-3′), reverse primer S1R (5′-GAAACGGTCTCTCCAACGCAT-3′) (Radha et al., 2013). The protocol for the thermal cycling was: 95 °C for 2 min, then 35 cycles of 95 °C for 30 s, hybridation at 58 °C for 30 s and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min.

For cyanobacteria, 16S rRNA genes were amplified by PCR with the forward primer 8F (5′- AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer 1351R (5′- GACGGGCGGTGTGTRCA-3′). The thermal cycling profile was 5 min at 95 °C then 25 cycles of 30 s at 95 °C, 30 s at 57 °C and 1 min at 72 °C, a final extension at 72 °C for 10 min.

The PCR product was sent to Genoscreen (France) for primer removal and for further sequencing using an automatic DNA sequencer (model 3730xl; Applied Biosystems). The nucleotide sequences were aligned with the closest relative sequences of representatives in the GenBank database by means of BLASTN (Basic Local Alignment Search Tool), an algorithm using the CLUSTALW program (Thompson et al., 2002) and available at the National Center for Biotechnology Information (NCBI). They were subsequently deposited in the NCBI database (refer to Table S2 accession numbers). DNA sequences blasted in NCBI were able to lead to both *Pseudostichoccus monallantoides* and *Diplosphaera* sp., and the microscopic observations of the cultures clearly displayed distinct algae morphologies (Fig. 2).

2.5. Biocidal assays

Four biocidal assays consisted of an accelerated biocolonisation of

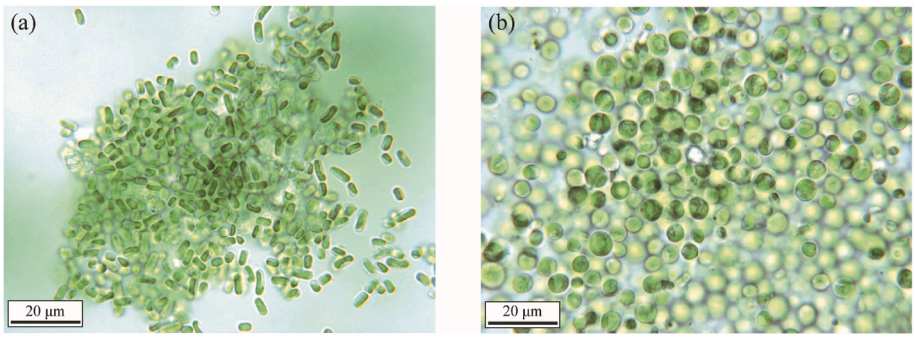

Fig. 2. Optical microscopy images of *Pseudostichococcus monallantoides* (a) and *Diplosphaera* sp. (b).

stone slabs in a climatic chamber which were carried out sequentially to adapt the protocol in function of the results and to evaluate biocides in progressively more drastic conditions. In assay 1, two different batches of slabs were inoculated with the algal colonizers of the bas-reliefs, *Chromochloris zofingiensis* and *Pseudostichococcus monallantoides*. Assay 2 consisted of increasing the quantity of biocidal product on slabs to optimize the efficiency. In Assay 3 and 4, the biocidal products were applied on water-saturated stones which represented more drastic and realistic conditions in which to evaluate the performance of biocides. Assay 3 consisted of the same biocolonisation protocol as Assay 2 but performed on stones saturated with water. Finally, Assay 4 evaluated the performance of the coatings on wet stone after a second inoculation of algae and cyanobacteria in order to be closer to the natural environmental conditions, where the seeding occurs continuously all the time.

The protocol of the accelerated biocolonisation consisted of inoculating slabs with a suspension of green algae isolated from biofilms growing on both bas-reliefs of the Pommery Champagne cellar. Algae were isolated and cultured in laboratory in stirred flasks with a liquid medium composed of sterile distilled water and Hoagland medium (Table S3). Cultures were incubated at 20 °C under a fluorescent light (Sylvania Gro-Lux-15 W lamp, PAR = 15 mmol (photons) m^{-2}. s^{-1}). An aliquot of algae was transferred every month into fresh medium.

For **assay 1 and 2**, algal suspension was diluted to obtain a similar concentration of around 50 cells/0.1 µL and checked by the chlorophyll *a* absorbance control at 665 nm using spectrophotometry (Thermo Fisher Scientific Genesys 10-S). Stone slabs were placed in Plexiglass cups; 2 mL of algal suspension was spread out on each stone surface and 20 mL of Hoagland medium was put at the bottom of samples after the inoculation and 5 mL of this solution was added regularly over the entire incubation period to ensure the wetness of the stones by means of capillary absorption.

For **assay 3**, algal suspension was diluted to get around 100 cells/0.1 µL and only 1 mL was spread on the slab's surface because stones were already saturated with water.

For **assay 4**, a mix of two algae (*Pseudostichococcus* sp. and *Diplosphaera* sp.) and a cyanobacteria (*Timaviella* sp.) were inoculated. An algal suspension was diluted to around 200 cells/0.1 µL, and 1 mL was spread on slabs and 400 µL of a cyanobacteria suspension was spread for a concentration of around 100 cells/0.1 µL.

The biocolonisation was carried out in a climatic chamber which displayed a constant temperature at 20 °C and 80% of relative humidity. Two neon lights (Sylvania Gro-Lux) released a PAR (Active photosynthetic radiation) between 20 and 30 µmol photons.m^{-2}.s^{-1} for 12 h every day. The stone and incubating conditions, the coating concentration and the algae and cyanobacteria inoculated on each assay are summarised in Table 1.

2.6. Evaluation of the biocidal effect

2.6.1. Colourimetry

The progressive greening of the stone surfaces, due to the algae biocolonisation, was followed by the measurement of the colour by a Chroma Meter CR-400 from Konica-Minolta with a light projection tube CR-A33c of 11 mm diameter. The calibration was performed with three flashes on a white ceramic plate CR-A43. Then 10 measurements were carried out on the upper face of every 5 × 5 cm sample before and after coating in the aim to estimate the colour variation of the stone impacted by coatings. Moreover, the algal growth during the biofouling assays was monitored by measuring the surface colour after the inoculation of the algae suspension (T0) and every week for the duration of the assay that means seven weeks for the first assay and five weeks for the other followed assays.

The device provides three parameters which are coordinates located in the CIELAB colour space (European committee for Standardisation, 2008). L* is lightness (0 = absolute black, 100 = absolute white), and a* and b* are the chromaticity coordinates located on the equatorial plane of the colour sphere: a* is the axis of green (a* < 0) and red/magenta (a* > 0); b* is the axis perpendicular to a* and it is the axis of blue (b* < 0)

Table 1
Summary of the conditions of the four assays.

Assay	Aim of the assay	Stone	Inoculated microorganisms	Incubation conditions	Concentration	
					POM-ILs	PR80
1	Evaluation of the biocidal coatings	Dry	*Chromochloris* sp. *Pseudostichococcus* sp.	7 weeks 20 °C 80% R.H.	0.1 g 0.2 g	0.2 g
2	Optimize the biocidal coating	Dry	*Pseudostichococcus* sp.	5 weeks 20 °C 80% R.H.	0.4 g	0.4 g
3	Effectiveness of the coating on wet stones	Wet	*Pseudostichococcus* sp.	5 weeks 20 °C 80% R.H.	0.1 g 0.2 g 0.4 g	0.2 g 0.4 g
4	Biocidal effect after re-inoculation	Wet	*Pseudostichococcus* sp. *Diplosphaera* sp. *Timaviella* sp.	5 weeks 20 °C 80% R.H.	0.1 g 0.2 g 0.4 g	0.4 g

and yellow (b* > 0).

For colour analysis, the mean was calculated for each slab (assay 1) or each triplicate (for the other assays) and the CIELAB lightness and chroma differences were calculated: ΔL*, Δa*, Δb*; they are between the mean at every week during the test period and the starting of the assay (T0). Δa* was chosen to monitor the stone greening during the biofouling assay and the global colour variation (ΔE^*_{ab}) was calculated before and after coating as follows (1) for the estimation of the coating impact on stone colour.

$$\Delta E^*_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

2.6.2. Chlorophyll a fluorescence

The chl. *a* fluorescence is non-invasive technique used to detect damages occurring in photosynthetic organisms before visible damages caused by natural environmental stresses or initiated by biocides. It is widely employed in every fields of biology (Van Kooten and Snel, 1990; Maxwell and Johnson, 2000; Lazár et al., 2006), aquatic pollutants (Choi et al., 2012; Dewez et al., 2018) and in cultural heritage to monitor the effectiveness of biocides used to eradicate stone dwelling phototrophs (Tomaselli et al., 2002; Tretiach et al., 2010; Pfendler et al., 2018b).

a) Monitoring with Junior PAM fluorometer

A handheld photosynthesis yield analyser, Junior PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany) was coupled to WinControl-3 Software, the device was used with the pulse-amplitude modulated (PAM) in combination with saturating pulse analysis of fluorescence quenching. Ten measurements were performed over the whole sample surface (25 cm^2), by means of a visible blue power LED (470 nm) for pulse modulated and saturating pulses. It is released with the fibre-optic probe of the fluorometer applied onto the surface. Samples were pre-conditioned half an hour in the dark, then the measurements were achieved in a room lighted by a green light which is not absorbed by plants. This environmental condition is required to measure the minimal (F_0) and maximal fluorescence (F_M) yields which were obtained with a saturating flash (1 s, 10 kHz). From these two parameters, the maximal quantum yield of Photosystem II, F_V/F_M, was calculated with formula (2). It is a reliable indicator of the maximum photochemical quantum efficiency (Baruffo et al., 2007) and has been used to investigate the biocidal impact of the coatings on the photosynthesis activity thus the physiological state of algae (Gladis et al., 2010; Manso et al., 2014; Dewez et al., 2018; Pfendler et al., 2018a; Pozo-Antonio and Sanmartín, 2018).

$$F_V/F_M = \frac{F_M - F_0}{F_M} \quad (2)$$

b) Monitoring with Imaging PAM fluorometer

Imaging PAM fluorometer (Walz, Effeltrich, Germany) was used at the end of the assay 4 in the aim to supplement Junior PAM which provided only F_V/F_M. Imaging-PAM was used to measure the kinetics of Chl. a fluorescence through the mapping heterogeneity in algal colonisation with the parameter Φ_{PSII} and the analysing fluorescence quenching, q_N and q_P outlined below, which provide information on plant adaptation to stress induced by biocides. The measuring system uses a fixed array of blue light-emitting diodes (LEDs) (peak wavelength = 470 nm) for saturating light pulses. The frequency of the pulses was adjusted to 10 Hz. Measurements were carried out at a distance of 11 cm between the camera and the slab's surface, corresponding to a 34 × 25 mm area. The image captured by the CCD camera was composed of 640 × 480 pixels. Slabs were previously conditioned in the dark for 30 min, the measured area was located on the central part of the slabs. The maximal fluorescence (F_M) yield was obtained with a saturating flash (1 s) as the device Junior PAM. The actinic light was then activated (PAR = 25 μmol photon.m^{-2}.s^{-1}, which was PAR released by fluorescence lights in the climate chamber) and finally a second saturating flash was triggered. Thereby the relative quantum yield of PSII (φ_{PSII}) was calculated as $(F_{M'}-F_S)/F_{M'}$, where F_S and $F_{M'}$ are, respectively, steady-state fluorescence and maximum fluorescence in the light. Photochemical quenching (q_P), which represents the photochemical energy conversion of PSII reactions centres when Q_A the electron acceptor of PSII is oxidised, and non-photochemical quenching (q_N) which indicates the energy dissipation through heat, were calculated according to (Van Kooten and Snel, 1990).

2.6.3. Statistical analyses

For the analyses of the biocidal assays, Δa* (colour parameter) and F_V/F_M (fluorescence parameter) were tested statistically using the non-parametric Mann and Whitney *U* test. Asterisk (*) indicates no statistically significant difference between control and coated slab measurements (n = 10 in assay 1 and n = 30 in assays 2, 3 and 4) at the 0.05 probability level.

3. Results and discussion

3.1. Evaluation of the colour change after application of the coatings

All biocides were tested as preventive treatments. Since they are applied as permanent coatings on the stones, they must have a minimum visual impact on the stone colour (Sasse et al., 1996). Here ΔE^*_{ab} of the dry chalk without coating was 1.5 ± 0.9 (CIELAB units) which displayed a natural colour variation (Fig. 3a). It was compared to ΔE^*_{ab} calculated from coated stones before and after every coating. For Assay 1 and 2, for which products were brushed on dry stones, ΔE^*_{ab} was equal to 2.6 and 2.9 for 0.2 g and 0.4 g of POM-IL1, respectively. Such low colour variation is below the generally established ΔE limit value ($\Delta E^*_{ab} \leq 3$ (Moreau et al., 2008; Burgos-Cara et al., 2017) and could not be detected with the naked eye. Stones coated with POM-IL2 had a lower colour variation, with $\Delta E^*_{ab} = 0.9$ and 1.4 for 0.2 g and 0.4 g, respectively. Preventol RI80 possessed the lowest colour variation, with $\Delta E^*_{ab} = 0.6$ for both quantities of liquid product. All ΔE^*_{ab} data are given in CIELAB units.

The coating applied on water-saturated stones (Assays 3 and 4) has led to higher colour variations. The colour variation produced by the POM-IL1 coating (ΔE^*_{ab} of 2.8, 2.7 and 2.3 at 0.1 g, 0.2 g and 0.4 g, respectively) was similar to the colour variation of the natural uncoated stones, with a ΔE^*_{ab} of 2.9 ± 0.7. The POM-IL2 coating displayed less colour variation, with ΔE^*_{ab} of 2.0, 1.9 and 2.6 at 0.1 g, 0.2 g and 0.4 g, respectively. On the other hand, the Preventol RI80 applied to wet stone led to the highest colour variation, with a ΔE^*_{ab} of 3.2 and 3.3 at 0.2 g and 0.4 g, respectively. Therefore, the colour variation depends on the humidity of the stone when the coating is applied, with POM-IL1 producing the greatest colour variation when applied on dry stones (Assays 1 and 2), followed by the POM-IL2 and the PR80. When applied on wet stones (Assays 3 and 4), PR80 is the product that shows higher colour variation, followed by POM-IL1 and POM-IL2. Crucially, the colour variation, $\Delta E^*_{ab} < 3$, confirms a low visual impact for all coatings, regardless the quantity and the condition of application.

3.2. Biocidal performance

3.2.1. Evaluation of biocidal coatings on dry stone over a seven-week period (Assay 1)

In this seven-week assay, the biocidal activity of POM-IL1, POM-IL2 and Preventol RI80 were evaluated against two algal strains isolated from the Pommery bas reliefs, *Pseudostichococcus monallantoides* and *Chromochloris zofingiensis*. Briefly, one control chalk slab (i.e. uncoated stone) was compared with slabs coated with POM-IL1, POM-IL2 or Preventol RI80, by inoculating each slab with *Chromochloris* sp. or *Pseudostichococcus* sp. (Fig. 4).

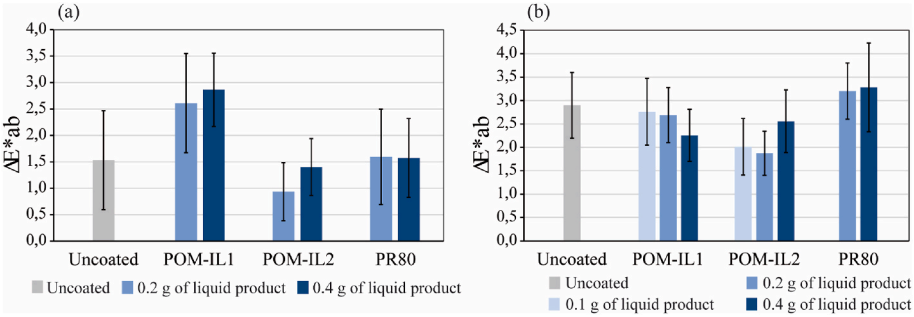

Fig. 3. Global colour variation (ΔE^*_{ab}) of natural uncoated stone compared with stones coated by POM-IL1, POM-IL2, and Preventol RI80 in dry (a) and wet conditions (b).

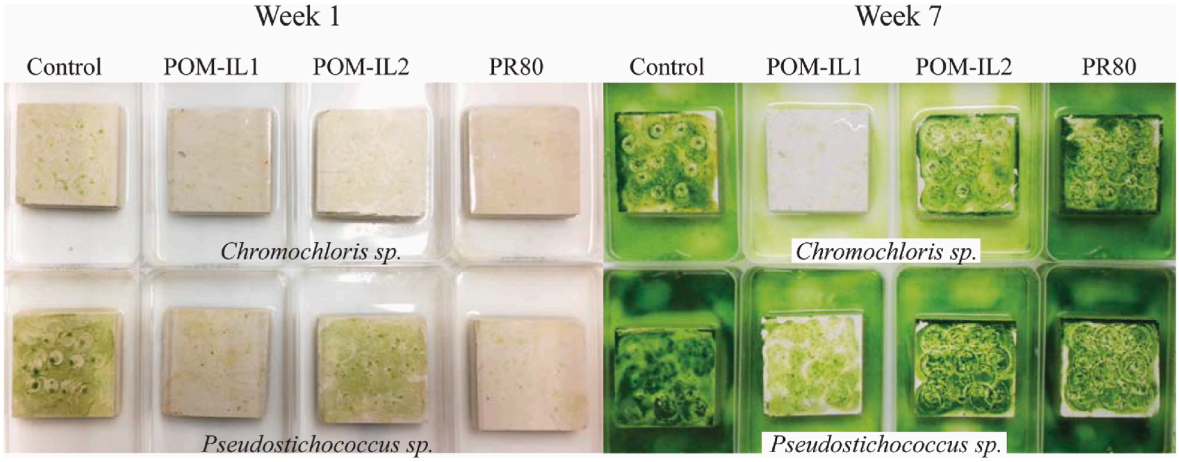

Fig. 4. Photos of inoculated controls and coated chalk slabs with *Chromochloris* sp. (upper slabs) and *Pseudostichococcus* sp. (lower slabs) at week 1 and week 7 of incubation.

After one week of incubation, the comparison of control stones colonised by algae displayed a net variation of growth between both algae (Fig. 5 a-b). The Δa* was −14.0 with *Pseudostichococcus* sp. whereas it was −3.3 with *Chromochloris* sp. The negative values showed a greening of stones for both algae because at the beginning of the incubation, all a* were between 0 and 0.3 for slabs inoculated with *Pseudostichococcus* sp. and between 0.4 and 0.6 for slabs inoculated with *Chromochloris* sp. At the same time of the assay, the strong negative values with *Pseudostichococcus* sp. indicated higher so quicker growth than with *Chromochloris* sp. For *Pseudostichococcus* sp. growth, Δa* had decreased to −20.2 by week 2 and remained low until week 7, -21.1. The *Chromochloris* sp. growth decreased progressively until reaching a value of −17.6 at week 5 and -14.6 at the end of the essay on week 7. Thus, the stones colonised by *Chromochloris* sp. were less green than by *Pseudostichococcus* sp. which supposed a slower growth with *Chromochloris* sp.

Nonetheless, the fluorescence parameter, F_V/F_M started at 0.28 with *Pseudostichococcus* sp. and 0.47 with *Chromochloris* sp. (Fig. 5c). Despite inoculating the slabs with the same number of cells, *Chromochloris* sp. had a higher maximum photochemical quantum yield than *Pseudostichococcus* sp. and thus a higher photosynthetic activity. Moreover, the maximum F_V/F_M value of 0.61 for *Chromochloris* sp. was reached at week 1, which was in accordance with results obtained with *Chlorella vulgaris* where the maximum value of F_V/F_M was reached at week 1 with 0.70 (Eyssautier-Chuine et al., 2015). The maximum F_V/F_M value of 0.62 for *Pseudostichococcus* sp. was measured at week 2 (Fig. 5d), which was later than the maximum of F_V/F_M for *Chlorella vulgaris*. The maximum of fluorescence of *Chromochloris* sp. seemed closer to *Chlorella vulgaris* than that of *Pseudostichococcus* sp. This result could be surprising since *Pseudostichococcus* sp. belongs to Trebouxiophyceae class like *Chlorella vulgaris* and *Chromochloris* sp. has a more distant taxonomy. Moreover, data from the two algae seemed similar to results obtained with *Dunaliella tertiolecta* where F_V/F_M was 0.6–0.7 but only after 5 days (Young and Beardall, 2003).

Pseudostichococcus sp. was slower than *Chromochloris* sp. at reaching a similar maximum photochemical efficiency despite showing a faster greening of slabs. After the maximum photochemical efficiency was achieved, F_V/F_M decreased gradually for both algae until reaching values of 0.39 for *Chromochloris* sp. and 0.44 for *Pseudostichococcus* sp. at the end of the assay. The decrease of PSII activity could be explained by the ageing of the algae with steady senescence of the first algae (Eyssautier-Chuine et al., 2015).

Accordingly, despite *Chromochloris* sp. having a maximum quantum yield higher and quicker than *Pseudostichococcus* sp. at the start of the assay, the greening of stones had been earlier and more intense with the *Pseudostichococcus* sp. algae, which is commensurate with a more rapid proliferation.

The two different POM-IL coatings displayed different impacts on algae. The Δa* for was between 0.35 and −0.94 and displayed nearly no greening of slabs by *Chromochloris* sp. during the whole assay. The photosynthetic activity of algae increased only at week 1 with F_V/F_M at 0.58 then it fell strongly and remained low until the end of the assay. Therefore, POM-IL1 had a strong biocidal impact on the *Chromochloris* sp. growth. With *Pseudostichococcus* sp., Δa* was close to 0 until week 2 whereupon it decreased to −0.82 (at week 3), suggesting greening, which decreased until Δa* equalled −9.96 at week 7. F_V/F_M started at 0.28 and decreased gradually until reaching 0.14 at week 3 which suggested a weaker photosynthetic activity and thus inhibition of algal growth. Nonetheless, from week 4, F_V/F_M increased from 0.22 to 0.35, indicating a resumption of the algal proliferation. Thus POM-IL1 was efficient during the first three weeks, it seemed less efficient against

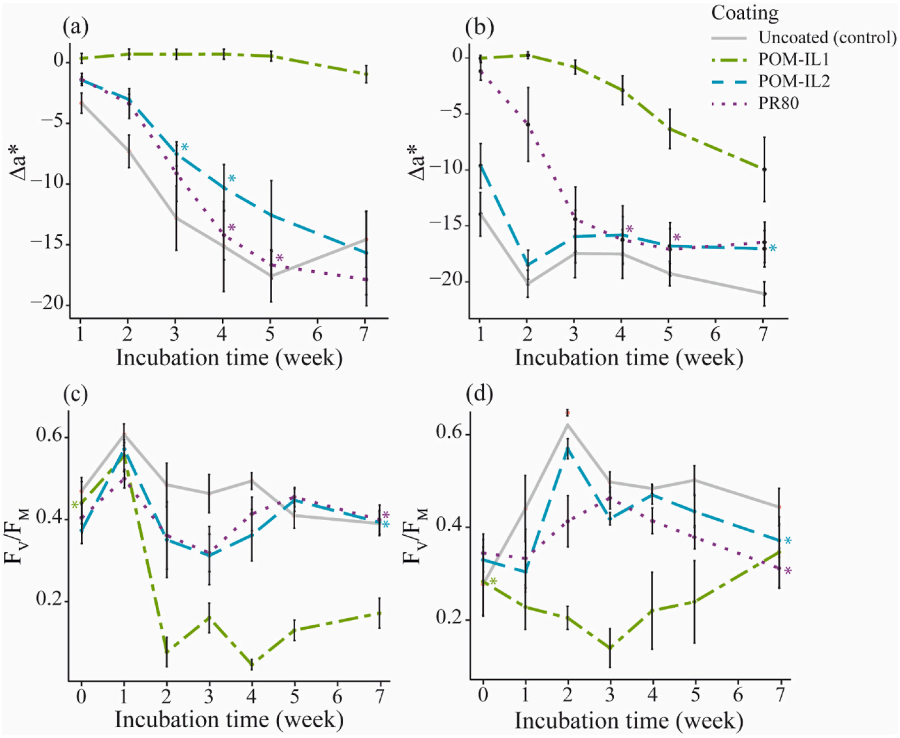

Fig. 5. Greening monitored by the colour variation Δa* and chlorophyll fluorescence F_V/F_M monitored on controls and coated stones inoculated by *Chromochloris* sp. (a–c) and *Pseudostichococcus* sp. (b–d) during 7 weeks of incubation. * indicates no statistically significant difference between control and coated slab measurements (n = 10).

Pseudostichococcus sp. than against *Chromochloris* sp. nonetheless the results might have been similar if *Chromochloris* sp. grew faster than *Pseudostichococcus* sp.

Results of the colonisation of *Chromochloris* sp. on POM-IL2 coated stones showed how Δa* was negative at week 1 (−1.45) suggesting an early greening of stones which decreased until Δa* was equal to −15.66 at week 7. Meanwhile, F_V/F_M started at 0.38 and increased at 0.57 after just one week of incubation, the rise of the maximum photochemical quantum yield showed a strong photosynthetic activity and a net growth of algae. Nonetheless F_V/F_M decreased at weeks 2 and 3 (0.35 and 0.31, respectively) indicating an inhibition of microorganisms by POM-IL2 but this decline was also observed for controls. Moreover, F_V/F_M increased from week 4–7 and was found to be 0.40, the same as the control slabs. Thus POM-IL2 had a short effect on the photosynthesis of *Chromochloris* sp. The colour variation Δa* monitored on stones inoculated with *Pseudostichococcus* sp. showed a similar trend to the controls and the chalk slabs were green already at week 1 (−9.64), decreased rapidly to −18.47 at week 2 and remained weak until the end of the test. This fast greening was highlighted by a rise of the maximum photochemical quantum yield at week 2 with F_V/F_M at 0.57. These values then decreased to between 0.35 and 0.40 over the remainder of the test period, indicating that POM-IL2 did not disturb the photosynthetic activity of *Pseudostichococcus* sp.

The biocidal effect of Preventol RI80 on chalk slabs inoculated with *Chromochloris* sp. was poor. Greening began at week 1 with a Δa* at −1.38 and continued to decrease over the test period and was measured to be −17.87 at week 7, thus the greening was as intense as that of controls. The photosynthetic activity of *Chromochloris* sp. followed a similar trend to POM-IL2 coated stones, F_V/F_M reached a maximum of 0.50 at week 1 like all incubated stones, controls and coated stones, then it decreased at weeks 2 and 3 to 0.36 and 0.32, respectively. The short-lived antiproliferative effect of Preventol RI80 during these two weeks did not last since values became as high as POM-IL2 and controls by week 7. However, the effect of Preventol RI80 on the *Pseudostichococcus* sp. growth was different to POM-IL2. Δa* was weakly negative (−1.20) that supposed a weak greening whereas with POM-IL2 it was already at −9.64 thus the slabs were greener. At weeks 2 and 3, Δa* decreased at −5.95 and −14.40 that displayed a greening of slabs but it remained weaker than POM-IL2 slabs at the time. Then it still decreased to the end of the assay with −16.47 at week 7 which was similar to POM-IL2 results. The maximum photochemical quantum yield, F_V/F_M, was weak and constant at 0.33 the first week. This stagnation of photosynthetic activity suggested a lack of algal growth and a biocidal action of Preventol RI80 but it did not last since F_V/F_M increased up to 0.46 which was a maximum at week 3 and showed a resumption of the algae development. Preventol RI80 had a better biocidal effect than POM-IL2 but not enough to avoid the greening of stones which were as green as POM-IL2 stones from week 4–7.

Consequently, POM-IL1 had an efficient biocidal impact on both species of algae over the seven-week test period; unlike POM-IL2 and Preventol RI80 which had a shorter biocidal effect on both algae. These results indicated that the biocidal effect could be improved by increasing the amount of product applied. Therefore, the second assay aimed to determine an optimal amount of POM-IL1 and improve the biocidal effect of POM-IL2 and Preventol RI80.

3.2.2. *Optimising the biocidal effect of the coatings (Assay 2)*

Pseudostichococcus sp. was the only algae used for the assay 2 due to its faster growth on chalk slabs, compared with that of *Chromochloris* sp.. With the aim of optimising the biocidal effect of all coatings, the amount of biocide treatment was increased to 0.4 g of liquid solution on triplicates used for each coating and control. Slabs were incubated in a climatic chamber for five weeks (Fig. 6).

After the inoculation, results of colour displayed a weak positive a* which was at 0.5 for controls, 0.6 for POM-IL1 slabs and 0.5 for POM-IL2 and PR80 slabs. After one week of incubation, the calculation of Δa* showed a net greening of control slabs which started with a Δa* at −2.1 then a strong negative Δa* (−15.7) at week 2 showed the definitive

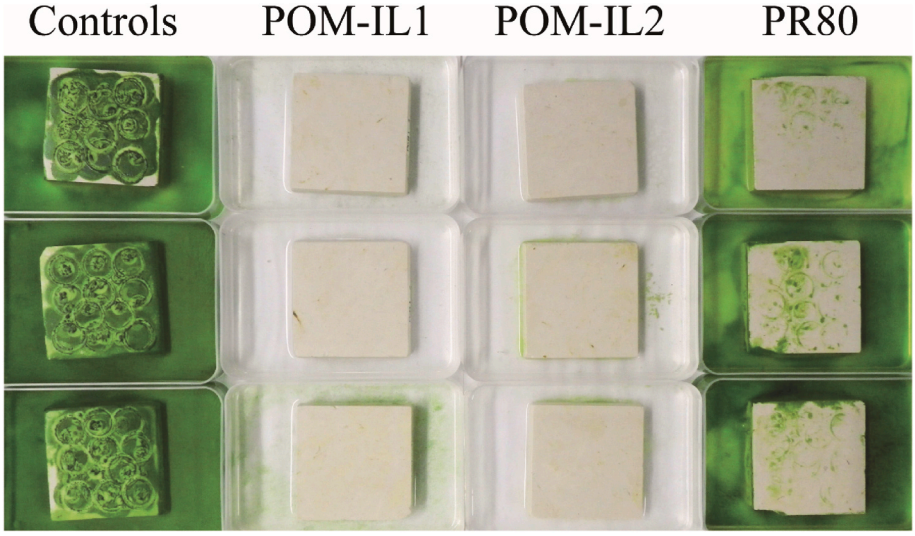

Fig. 6. Photos of inoculated triplicates of controls and coated slabs with *Pseudostichococcus* sp. at week 5 of incubation.

settlement of algae (Fig. 7a). This was corroborated by the maximum photosynthetic activity, F_V/F_M, that highly increased during the two first weeks (Fig. 7b). Thereafter, as greening intensified, Δa* continued to decrease until reaching a value of −19.1 after five weeks. On the other hand, POM-IL1 and POM-IL2 showed constant Δa* close to 0 throughout the duration of the five-week assay, indicating no greening of coated slabs. Moreover, F_V/F_M, started at 0.15 and 0.12 respectively for POM-IL1 and POM-IL2; it decreased from week 1 at 0.06 and 0.05 and remained close to 0 to the end of the assay which revealed the strong inhibition of the photosynthetic activity of algae by both coatings. For slabs treated with Preventol RI80, the colour variation Δa* was measured to be between 0.2 and 0.4 indicating no greening. The photosynthetic activity monitored by F_V/F_M decreased progressively from 0.14 at the starting of the assay to 0.02 at week 3 which revealed an inhibition of the photosystem II in the photosynthetic chain. Nonetheless, F_V/F_M increased during weeks four and five and Δa* was negative, indicating a resumption of algae growth. The biocidal effect of Preventol RI80 was effective for only three weeks, despite the good anti-biocolonisation preventive results of benzalkonium chloride for a longer time as two months in an accelerated growing assay with a mixing of green algae and cyanobacteria (Barriuso et al., 2017). Moreover, Preventol RI80 was efficient on cultural heritage to remove fungi and bacteria (Urzì et al., 2016; Favero-Longo et al., 2018; Li et al., 2020; Rugnini et al., 2020) as well as algae and lichens (Nugari et al., 2009; De los Ríos et al., 2012; Pfendler et al., 2018b). Furthermore, many studies have demonstrated antimicrobial activity for a curative action in a short time in most of case and in a longer time when lichen was previously soaked for 1 h in Preventol RI80 (Coutinho et al., 2016; Vannini et al., 2018). A long-term efficiency of this biocide probably needs a more significant amount whereas POM-ILs performed with a lower amount. Nonetheless, the performance of any biocide can vary significantly as a function of the concentration and amount of product that is applied, which furthermore depends on the absorption of this product by the surface substrate material.

3.2.3. *Application of the coatings to wet stone (Assay 3)*

The aim of this assay was to evaluate the application of the biocides on wet stone, which replicates the more drastic natural environmental conditions of the Pommery cellar. Such conditions can limit the performance of the biocidal coatings due to a number of factors, including poor substrate penetration and polymerisation. In this assay, the chalk slabs were inoculated by *Pseudostichococcus* sp. in the same conditions as assay 2 (Fig. 8).

After the inoculation of algae, a* was between 0.2 and 0.8 and the calculation of Δa* for the control slabs showed significant greening after five-week incubation period, where Δa*changed from −7.0 to −19.2. For the highest amount of coating (0.4 g), Δa* remained close to 0 over the test time for every coating and the photosynthetic activity F_V/F_M of *Pseudostichococcus* sp. remained low (between 0.02 and 0.14) demonstrating good biocidal activity for every coating (Fig. 9a and b).

With 0.2 g of product on slabs, POM-IL1 and POM-IL2 Δa* were also close to 0, therefore no greening appeared during the assay. In addition,

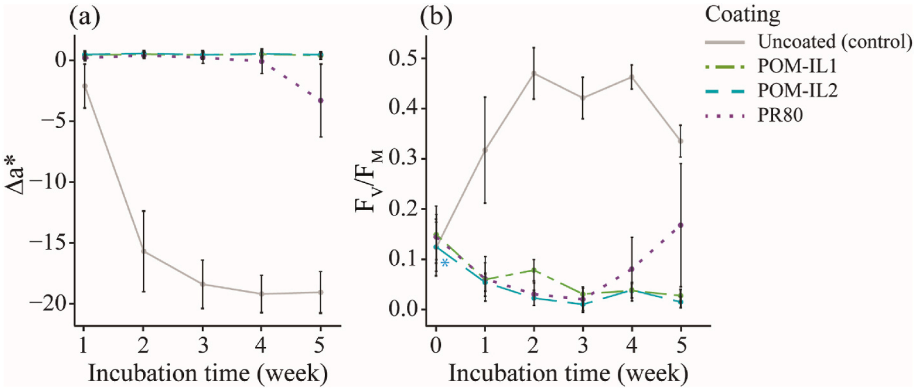

Fig. 7. *Pseudostichococcus* sp. growth on control and coated slabs over five weeks monitored by the colour variations with Δa* (a) and the chlorophyll fluorescence F_V/F_M (b). * indicates no statistically significant difference between control and coated slab measurements (n = 30).

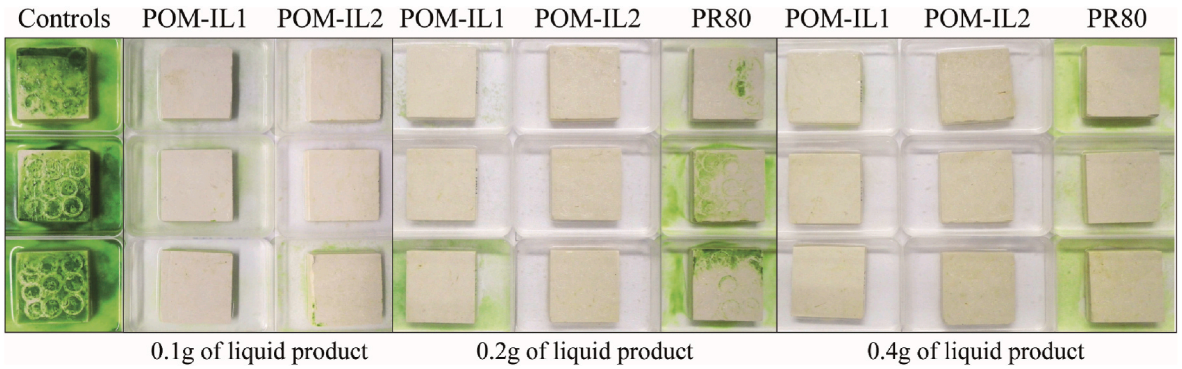

Fig. 8. Photos of inoculated triplicates of controls and coated slabs 5 weeks after inoculation with *Pseudostichococcus* sp.

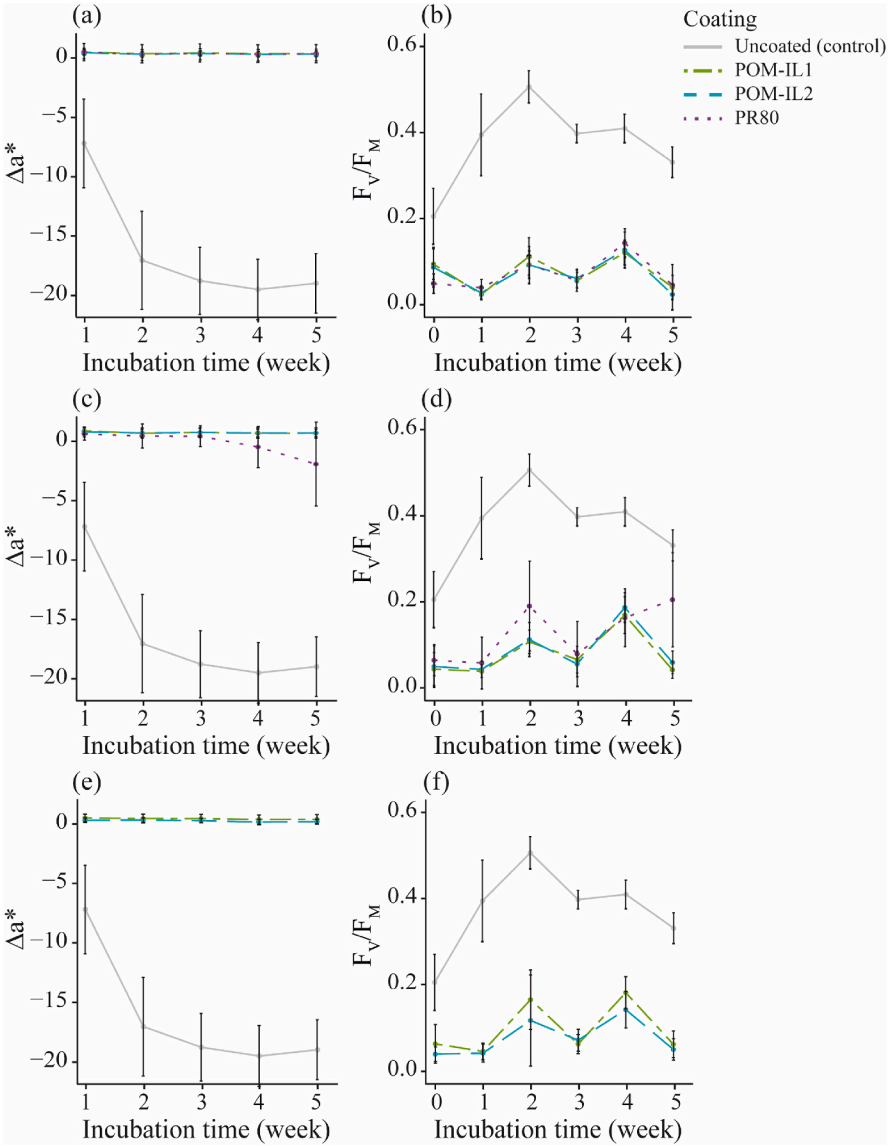

Fig. 9. *Pseudostichococcus* sp. growth during the five-week assay monitored by the colour variation Δa* and the chlorophyll fluorescence F_V/F_M on wet stones brushed with 0.4 g (a–b), 0.2 g (c–d) and 0.1 g of liquid products (e–f).

F_V/F_M remained low between 0.04 and 0.19 which displayed a weak photosynthetic activity of algae and no real growth (Fig. 9c and d). For slabs coated with Preventol RI80, Δa* was positive and close to 0 like the other coated slabs but at week 4, it was negative (−0.8) and still decreased at −2.7 at the last week which meant the greening settled on slabs (Fig. 9c). Meanwhile, F_V/F_M remained low for all coated slab, however, at week 5, the Preventol RI80-coated slabs increased to 0.20 suggesting a resumption of photosynthetic activity; whereas F_V/F_M

remained weak for POM-IL-coated slabs (0.06). All coatings had a net biocidal effect compared to controls, but the greening developed with Preventol RI80 at the end of the assay, showing less biocidal effect of this compound in comparison with the POM-ILs coatings (Fig. 9d).

The monitoring of the colonisation of slabs coated with 0.1 g of POM-IL1 and POM-IL2 displayed a lack of greening through Δa* which remained close to 0, from 0.7 to 0.5 for POM-IL1 slabs and from 0.4 to 0.3 for POM-IL2 (Fig. 9e). This result was confirmed with a weak maximal photochemical efficiency of the PSII for all the duration of the assay, between 0.05 and 0.18 for POM-IL1 slabs and between 0.04 and 0.14 for POM-IL2 (Fig. 9f). Thereby, the low chlorophyll fluorescence of algae revealed the inhibition of the PSII in the photosynthetic chain by biocides that led to a lack of the algal growth over the five weeks of the assay.

Consequently, both POM-IL1 and POM-IL2 had a net biocidal impact on algae even under drastic application conditions as water saturated stones which avoided the penetration in the porosity and limited the amount of product to be applied. It is worth noting that the application of 0.2 g and 0.4 g of liquid POM-ILs only on the surface has indeed generated a thicker coating which did not change the stone colour but it was visually detectable by a glossy aspect. Therefore, the lower amount of POM-ILs at 0.1 g is preferable since it has not been detected by naked eye and it kept a good biocidal performance.

3.2.4. *Re-inoculation of coated slabs with algae and cyanobacteria (Assay 4)*

The aim of the final assay was to simulate natural environmental conditions by reusing coated slabs which gave a good biocidal performance in a previous assay and seeding them with a second inoculation of algae and cyanobacteria (Fig. 10).

For this last assay, Chl. *a* fluorescence was monitored every week with Junior PAM to calculate F_V/F_M then, at the end of the assay, Imaging-PAM was used for mapping heterogeneity in algal colonisation with the parameter Φ_{PSII} and for analysing fluorescence quenching, q_N and q_P, as previously described. The fluorescence mapping highlighted a gap between the fluorescence signal which did not match with the biological growth (Fig. 11). This result was induced by a gap analysis of the cyanobacterial fluorescence. The pulse-modulated excitation LED lamps from Imaging-PAM with a peak emission at 470 nm was only adapted to the main fluorescence from chlorophyll pigments in land plants and algae. Those pigments mostly absorb blue light (around 400 and 480 nm) and red light (around 650–700 nm) whereas in cyanobacteria, the principal light-harvesting complexes are phycobilisomes which absorb mainly yellow/orange light (around 500–680 nm) (Schreiber et al., 1995; Campbell et al., 1998; Ogawa et al., 2017). In consequence, Chlorophyll fluorescence analysis did not display the fluorescence emitted by the cyanobacteria growth but only the fluorescence from algae.

As a result, a* measured after the inoculation was weakly positive, between 0.3 and 0.6. The monitoring of the controls displayed a greening through Δa* started the first week with −4.8, but this decreased drastically to −18.5 at week 2 and there was a strong greening which remained visible until the end of the assay (Fig. 12a). Photosynthetic activity of algae was monitored on controls as a regular algal growth on stones, through the chl. fluorescence every week with F_V/F_M. It started at 0.14 and reached a maximum value of 0.58 at week 3 (Fig. 12b). The calculation of the other parameters from the mapping of the fluorescence at week 5, showed the effective quantum yield, Φ_{PSII}, was 0.44 and similar for the three slabs considering a very minor standard deviation (0.01). q_P was 0.84 which was high and reflected a high electron flow away from PSII for the photochemistry and the reaction centre opening whereas q_N is weaker (0.39) which displayed a thermal dissipation of excess energy (Fig. 13b).

On PR80-treated slabs, the colour variation was very similar to controls with the decrease of Δa* over time (from −3.89 to −16.15), thus the algal growth has not been avoided by the biocide. Moreover, F_V/F_M increased progressively from 0.17 at week 0–0.56 at week 5 which displayed a photosynthetic activity as effective as that of controls but the effective quantum yield (Φ_{PSII}) was 0.34, so weaker than controls (0.44) which further confirmed the ineffectiveness of PR80 on the algal growth (Fig. 13a). Moreover, the increase of q_N at 0.87 showed heat dissipation which reflected a stress induced by the biocidal effect.

On POM-IL1 treated stones, the colour variation remained close to 0 for the three batches, which suggested no greening during the assay. A slight decrease was detected at week 5 for POM-IL1 at 0.1 g and at 0.2 g where Δa* were respectively −0.30 and −0.35, compared to POM-IL1 at 0.4 g which remained stable. The chl. fluorescence showed that F_V/F_M for POM-IL1 throughout the duration of the assay was between 0.01 and 0.18, which was very low compared to the control samples. In consequence, POM-IL1 had a real long-term biocidal effect despite the wet condition of application on stones. In detail, Φ_{PSII} images were black because the detection of the fluorescence was very weak, 0.08, 0.17 and 0.07 respectively for 0.1 g, 0.2 g and 0.4 g, considering few algae remained alive. Nonetheless, q_P was substantial with 0.67 for 0.1 g and 0.2 g POM-IL1 and 0.50 for 0.4 g which indicated a photosynthetic activity with the electron transfer and the opening of the PSII centres, but the high deviation standards suggested a big heterogeneity of the fluorescence signal from triplicates. In addition, the thermal dissipation, q_N was 0.33 for 0.4 g POM-IL1 and showed that the increase of POM-IL1 on stones generated some form of oxidative stress on the algae cells.

Results of the colonisation of slabs treated with POM-IL2 were more moderate. With a quantity of 0.1 g of product on slab, Δa* started

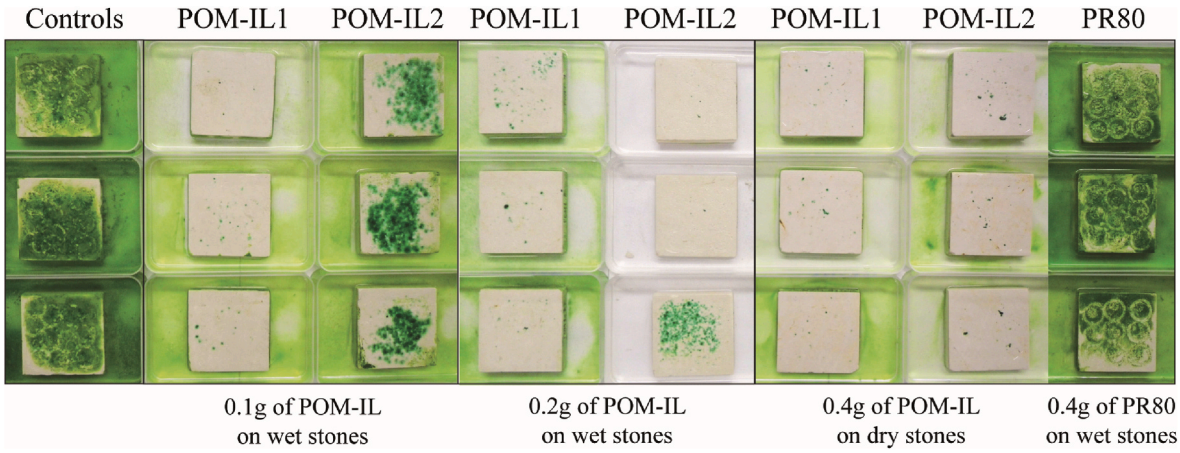

Fig. 10. Photos of inoculated triplicates of controls and coated slabs 5 weeks after inoculation with two algae (*Pseudostichococcus* sp. and *Diplosphaera* sp.) and a cyanobacteria (*Timaviella* sp.).

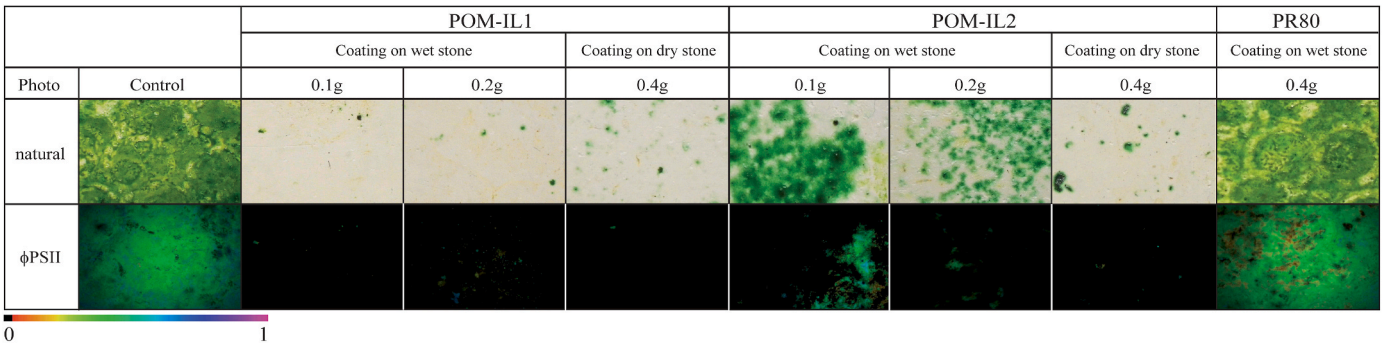

Fig. 11. Natural and Chl. fluorescence (φ_{PSII}) imaging of one stone at 5 weeks of inoculation for control and coated stones.

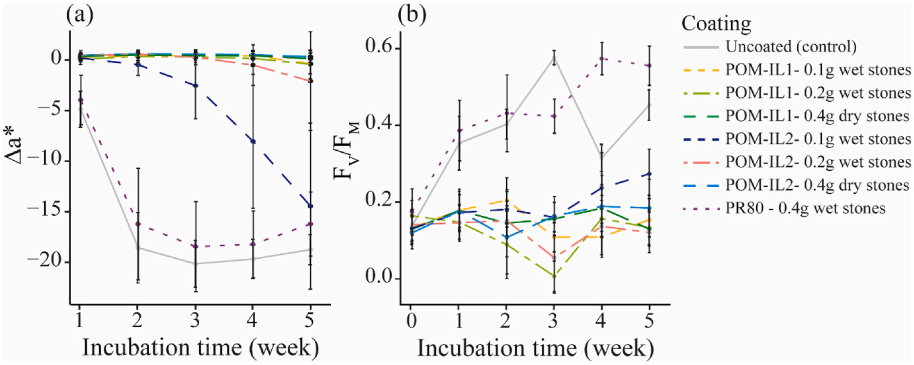

Fig. 12. Algal and cyanobacterial growth monitored during 5 assay weeks on treated stones and controls through the colour variations with Δa* (a) and F_V/F_M measured the chlorophyll fluorescence of the algal growth (b).

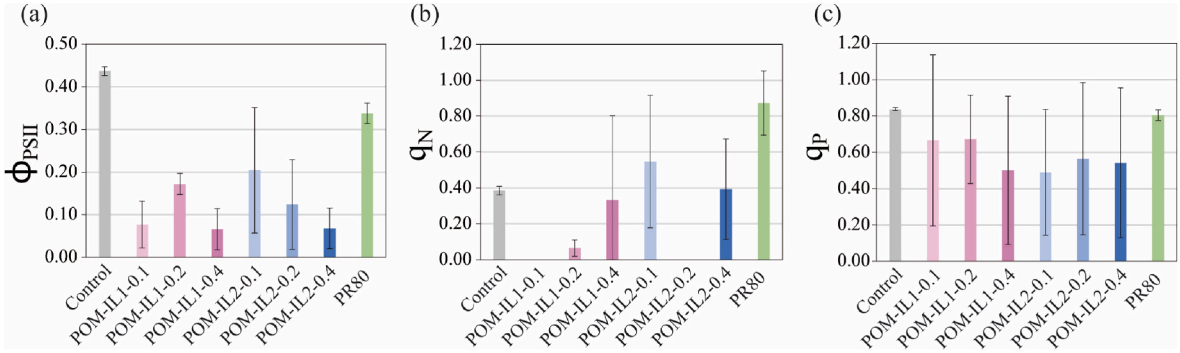

Fig. 13. Histogram plots of mean and standard deviation of φPSII (a), q_N (b) and q_P (c) as Chl. fluorescence parameters measured at 5 weeks after inoculation in controls and stones coated with 0.1 g and 0.2 g of product in wet condition and 0.4 g in dry condition (n = 3).

positive with 0.26 at week 1 then it was negative from week 2 with −0.41 and decreased week-on-week thereafter until −14.38 at week 5. Thus, stones were progressively green and biocolonised by both algae and cyanobacteria. With a quantity of 0.2 g of product on slab, Δa* was positive from week 1 to week 3 then it started to decrease and to be negative at week 4 with −0.44 and at week 5 with −2.03. With 0.2 g of product, there was a delay of greening, POM-IL2 had a better efficiency. For the last batch with 0.4 g of product, Δa* remained positive from 0.42 to 0.19 during the whole duration of the assay thus no greening was detected on stones. POM-IL2 with 0.4 g applied on dry stones was more efficient than a weaker amount of product applied on wet stones. The chl. fluorescence monitored with F_V/F_M every week, remained weak for all batches but for slabs with 0.1 g of POM-IL2, it increased slightly at week 4 and 5 with 0.24 and 0.27. Indeed, Φ_{PSII} images displayed a fluorescence signal which was lower than could suggest the real biological growth since it did not record cyanobacteria fluorescence, but it indicated a definite development of algae with a Φ_{PSII} of 0.20 but that remained lower than controls. The biocidal impact of POM-IL2 at 0.1 g has not been optimum but it led to a delay of the greening and a different biological growth where cyanobacteria rather developed than algae which were dominant on controls. Thus, rather the biocide seemed to act on algae than on cyanobacteria. Moreover, q_N was higher than control q_N; this increase of the heat dissipation by algae suggested a stress generated by the biocide. In addition, q_P was lower than controls which indicated a decrease of PSII primary photochemistry and of electron transport activity (Fig. 13c). At week 5, the effective quantum yield, Φ_{PSII} was of 0.20 with 0.1 g, 0.12 with 0.2 g and 0.07 with 0.4 g, thus it progressively decreased with the amount of POM-IL2 applied on stones despite the heterogeneity of values. This result supposed a stronger biocidal effect with the increasing of the dose. In consequence, despite POM-IL2 at 0.4 g applied on wet stones has not been tested a second time in assay 4, the result suggested that at 0.4 g of biocide on slabs, the

impact of POM-IL2 on the photosynthesis could have been more significant than at 0.2 g.

Finally, POM-IL1 and 2 were found to be more efficient than Preventol RI80 at avoiding a second biocolonisation of chalk stones by *Pseudostichococcus* sp., *Diplosphaera* sp. and *Timaviella* sp.. POM-IL1 possessed an optimal biocidal effect at 0.1, 0.2 and 0.4 g applied on dry and wet stones, and it proved its long-lasting efficiency in assay 4. POM-IL2 provided a poorer performance since its efficiency was optimal with 0.4 g of product on dry stones, but it failed for the second seeding with lower amount applied on wet stones. Thus, POM-IL1 outperformed POM-IL2 in more drastic experimental conditions.

Such results on POM-ILs are promising to eliminate lampenflora on bas-reliefs, nonetheless the recent environment of the Pommery Champagne cellar can be improved to prevent the biocolonisation. Artificial lighting promotes the growth of photosynthetic biofilms by the intensity, wavelength and temperature it generates (Mulec et al., 2008; Havlena et al., 2021), and it is the only parameter which can be improved in the context of Pommery Champagne cellar. The illumination equipment has changed for the two last decades and the gradual installation firstly aimed to highlight bas-reliefs by incandescent then halogen lamps. Recently the number of spots decreased and light emitted diode (LED) are lit temporarily to limit the amount of energy and the temperature increasing (D'Agostino et al., 2015). Nevertheless, contemporary art works are still exposed in the halls where bas-reliefs have been curved and need lights to be highlighted with tourists, some of them are even luminous and provide a weak but constant lighting to lampenflora which adapts to a weak light emission. Indeed, cyanobacteria and some eukaryotic algae have phycobiliproteins as pigments in addition of chlorophyll, which expand the absorption spectrum of primary pigments and enable phototrophic microorganisms survival even at low light intensities (Chaneva et al., 2007; Mulec et al., 2008; Baquedano Estévez et al., 2019). Consequently, the reduction of light intensity is not enough for a preventive strategy to limit lampenflora growth. Effort must be spent on the selection of lights whose wavelengths are less favourable for cyanobacteria like a band around 500 nm and above 700 nm (Bruno and Valle, 2017) and for overall photosynthetic organisms like 560 nm (Baquedano Estévez et al., 2019). Nonetheless, controlling the development of photosynthetic biofilms by using a spectral light emission the less adapted to the photonic assimilation performance of microorganisms was studied for the Nerja cave, by both empirical and theoretical methodologies, to select LED lighting systems limiting photosynthesis, but the variation of results between those methodologies and the in-situ data highlighted the complexity to find an adapted lighting system due to the presence of various biofilms made of a diversity of photosynthetic microorganisms with different pigmentary compositions and able to adjust the pigment content to a short shift in the light emission (Muñoz-Fernández et al., 2021).

4. Conclusions

The unique underground environment of the Pommery Champagne cellar leads to extended lampenflora biofilm growth on the UNESCO listed bas-reliefs carved in chalk in the cellar walls. These conditions are representative of a habitual problem in biodeterioration, where periodic cleaning is required to mitigate against poorly performing commercial biocides. The results of our study demonstrate that preventative biocidal treatments based on polyoxometalate-ionic liquids (POM-ILs) were more effective than commercial Preventol RI80 against algal strains isolated from the bas reliefs. One variant (POM-IL1) was capable of sustained prevention of biofilm growth when applied to wet chalk. It is important to note that while the microorganisms used in this study are representative microflora that were isolated from the bas-reliefs, our ongoing work is currently focussed on an in-depth evaluation of the microflora responses to these biocidal treatments applied *in situ* on the walls of the Pommery champagne cellar. Our current hypothesis is that the most effective strategy will involve combining this chemical biocide approach with UV-C irradiation as a multi-mode strategy to prevent biocolonisation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2022.105459.

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