Corso di dottorato nazionale Heritage Science - ciclo XXXIX

POL&INE: POLysaccharides and protEIN-based hydrogel from microalgae for cultural heritage

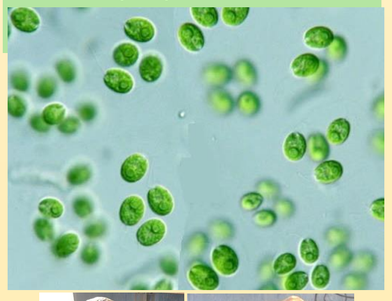
Giulia Gasperuzzo[1], Maria Grazia Ortore[2], Amina Antonacci[3]
[1]Department of Science of Antiquities, Sapienza University of Rome
[2]Departement of Life and Environmental Science, Marche Polytechnic University, Ancona
[3]Institute of Crystallography, National Research Council, Rome

Laboratory of Molecular Biophysics, DISVA

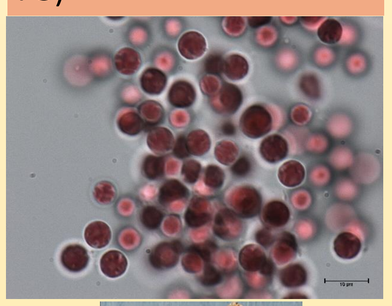
Introduction

The heritage conservation field progressively highlights and prioritizes actions and methods that have a significant positive impact on the environment and society. Integrating microbiology into cultural heritage preservation unlocks novel avenues for sustainable materials and techniques. Bio-based hydrogels present adaptable and environmentally sound choices. We have investigated polysaccharides derived from microalgae, due to their antioxidant and biodegradable qualities, and used them for new biomaterials whose aim is to restore paper [1]. In this context, this research aims to characterize a mixture of polysaccharides and proteins extracted from microalgae cultures using sustainable solvents [3] and to assess its potential as a consolidant and cleaning agent for paper-based artworks, when incapsulated into hydrogels. Freshwater strains of *Chlamydomonas reinhardtii* (CC125 and SAG 11-32b), along with marine species of *Porphyridium purpureum* sp. and *Lyngbya* sp., are chosen as established sources of polysaccharides and bioactive compounds exhibiting antifungal and antibacterial features.

Chamydomonas reinhardtii CC125 (C1) SAG11-32b

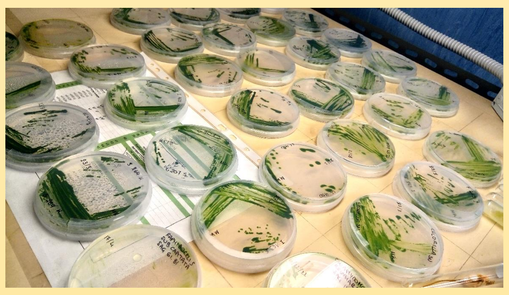


Porphyridium purpureum sp. (P7, EPS-P3)



Lyngbya sp. (LYN3)

Workflow



Culture's growth
- OD_{750}
- Cells count
- Chlorophyl and pigment
- Fluorescence efficiency

Mixture extraction
- Hot water extraction
- Precipitation using ethanol
- Lyophilization

Extract characterization
- Quantification of proteins and total reducing sugars
- FTIR, UV
- DLS
- GC-MS
- Antioxidant capacity

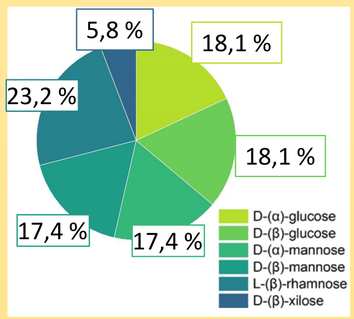
Paper application
- Extract applied as a consolidant by brush
- Hydrogel charged as a cleaning agent (agar, gellan gum) [3]

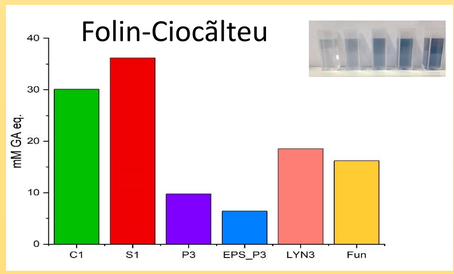
Extracts characterization

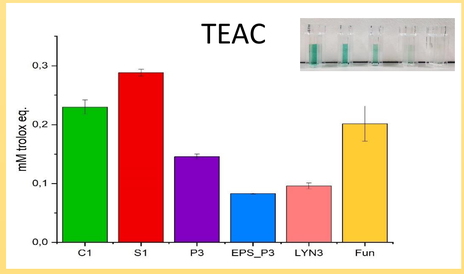
To characterize extracts' composition, we analysed them by means of:
- FTIR and UV/VIS spectroscopy;
- Bradford assays to quantify the protein contents and Dubois assay to measure the total amount of reducing sugar;
- GS-MS to detect the monosaccharides;
- CHNSO elemental analysis;

By TEAC method we evaluated the antioxidant capacity, and by Folin-Ciocalteu approach the total polyphenols' content.

Sample	N (%)	C (%)	H (%)	S (%)
C1	8,7	33,6	5,4	0
S1	11,1	32,2	6,0	0,4
P7	2,8	11,8	2,4	10
ESP_P3	0,1	3,9	2,9	15,3
LYN3	1,2	13,8	2,7	7,1

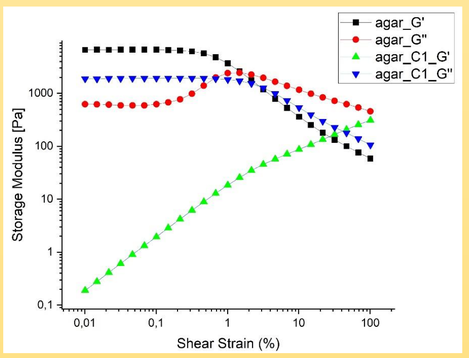






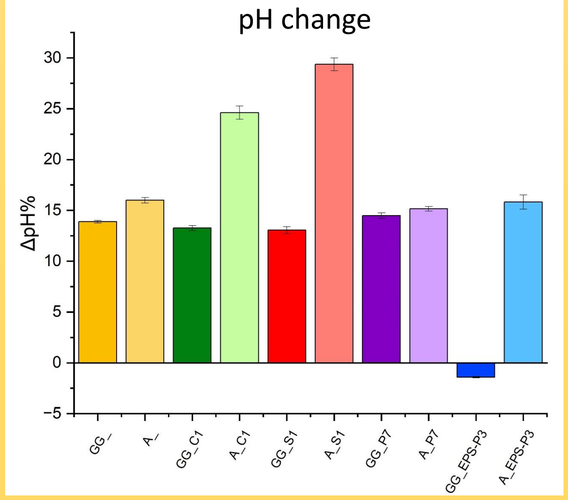
To create a hydrogel, whose properties should reproduce those of Funori, we used:
- agarose;
- gellan gum.

The amplitude sweep tests show how the crosspoint G'/G'' is at higher shear strain when the agarose hydrogel is charged with the microalgae extracts. Hence, the extracts increase the stability of the gel under shear stress, as it increases the Linear Viscoelastic Region (LVR) as well. Further measurements are in progress to confirm these results for all the extracts.

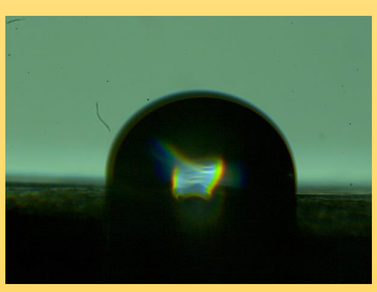


Extracts applications on paper

- **Consolidant** treatments → the extracts were applied to the specimens via brush, utilizing aqueous solutions with pH values of 7 and 13. The higher basic pH was employed to mitigate paper acidification and establish an alkaline reserve. To assess the efficacy of the treatment and compare treated and untreated samples, the contact angle method was utilized. The two extracts derived from *Chlamydomonas r.* exhibited a favorable effect, yielding contact angles exceeding 90°
- **Cleaning** agent → charged hydrogels are currently under investigation as cleaning agents. These hydrogels are being applied to the aged paper specimens, both with and without the incorporation of the extracts, and the resultant change in pH is being measured to evaluate the product's performance. Preliminary findings suggest that agarose hydrogels demonstrate superior efficacy compared to gellan gum hydrogels. Notably, the extracts from *Chlamydomonas r.* (C1, S1) induced the most substantial pH variation, increasing the value from 4.5 to 6.0



	pH 7	pH 13
C1	114,6°	64°
S1	118,8°	37,4°
P7	68,2°	50,6°
EPS-P3	61,5°	53,1°
Funori	77,3°	



Next steps

- Investigation of hydrogel's structure by means of DLS and SAXS, and of its mechanical properties by rheological measurements.
- Application of the hydrogel on paper specimens, and study of its stability by means of cycles of artificial ageing. The deterioration will be studied by means of colorimetric measurements, UV/VIS/NIR imaging, optical and electronic microscopy, SANS analysis to evaluate paper hydration and porosity.

References

[1] M.R. Caruso, *et al.*, J Mater Sci 58,**2023**, 12954–12975 https://doi.org/10.1007/s10853-023-08833-5
[2] A. Masi, *et al.*, Carbohydrate Polymer Technologies and Applications, **2025**, *Vol. 9*. https://doi.org/10.1016/j.carpta.2025.100713.
[3] C. Mazzuca, *et al.*, *Journal of Colloid and Interface Science* 416, **2014,** 205-211 https://doi.org/10.1016/j.jcis.2013.10.062