Corso di Dottorato Nazionale in Heritage Science

Università Politecnica delle Marche Ciclo XXXIX

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

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Our aim is the development of an innovative and sustainable product of organic origin, to meet the needs of the cultural heritage market field. The output of our research is designed for the conservation of paper-based artwork and is capable of respecting both the environment and the operator, and its production must be green. Hence, our research focuses on a hydrogel, based of a mixture of polysaccharides and proteins, extracted from microalgae culture, which will be tested for paper repairment and/or conservation.

The great potential of microalgae lies in their versatility and ability to produce natural compounds, such as polysaccharides, proteins and pigments. For our aims we choose strains which are well-known sources of these compounds. Sulphate polysaccharides, with interesting properties as antioxidant and antifungal, should be the main part of the right material for the hydrogel production. The polysaccharides are present as component of the cell walls (endopolysaccharides) and dissolved in the growth's medium (esopysaccharides, EPS).

Workflow

- Optical density (OD750)
- Cells count
- Chlorophyl and pigment
- Fluorescence efficiency

Culture's growth

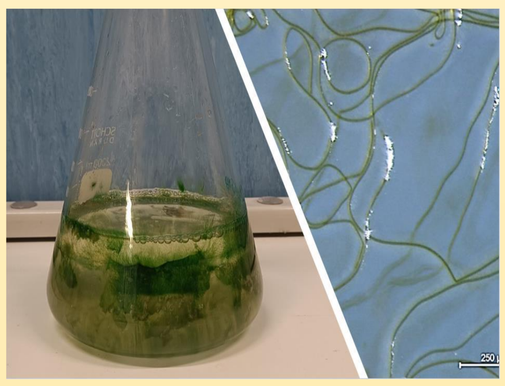
- Hot water extraction
- Precipitation using ethanol
- lyophylization

Mixture extraction

- Quantification of proteins and total reducing sugars
- Spectroscopy (FTIR, UV)
- Dynamic Light Scattering
- GC-MS

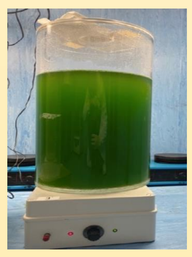
Mixture's characterizazion

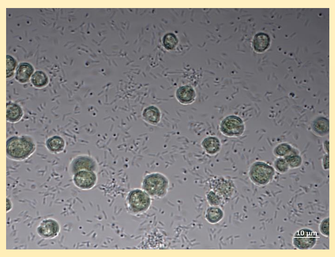
Lyngbya sp.



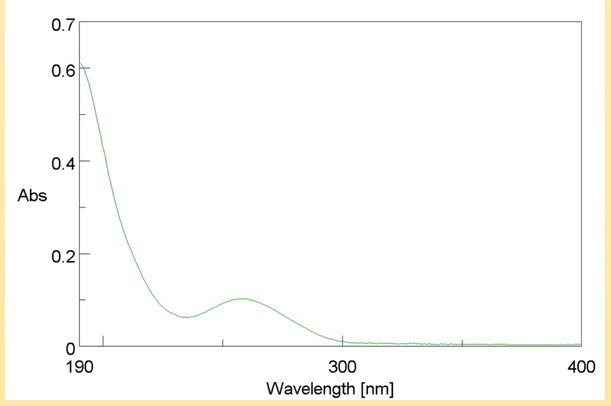
Porphyridium purpureum sp.

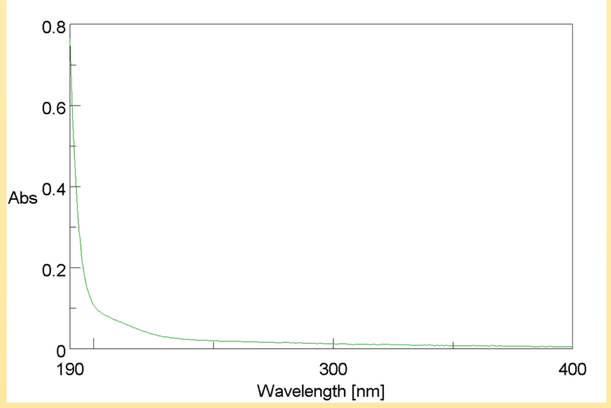
Chlamydomonas reinhardtii (CC125 and SAG 11-32b)



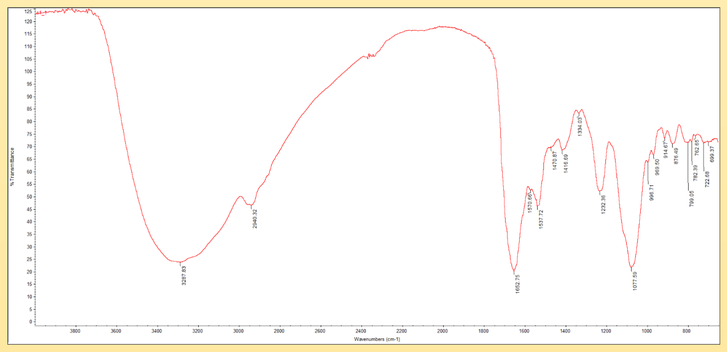


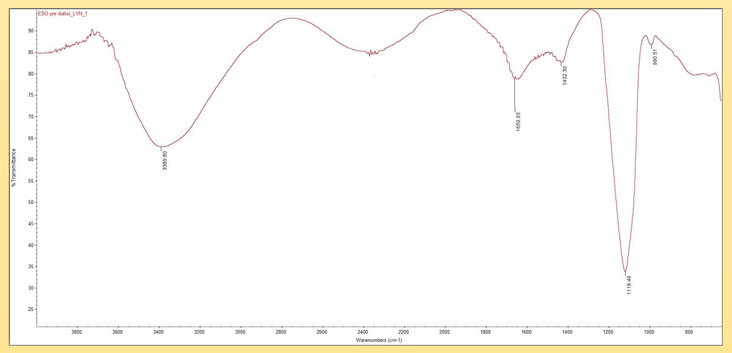
Results





In the UV range, the sample of endopolysaccharides (left) extracted from *Porph*. shows the absorbance of carbohydrates at 190 nm and proteins or polypeptides at 260 nm. While the sample of esopolysaccharides (right) shows only the absorbance of the carbohydrates

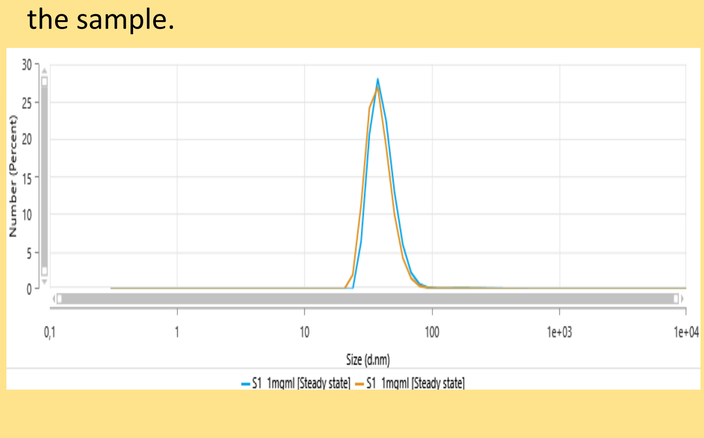


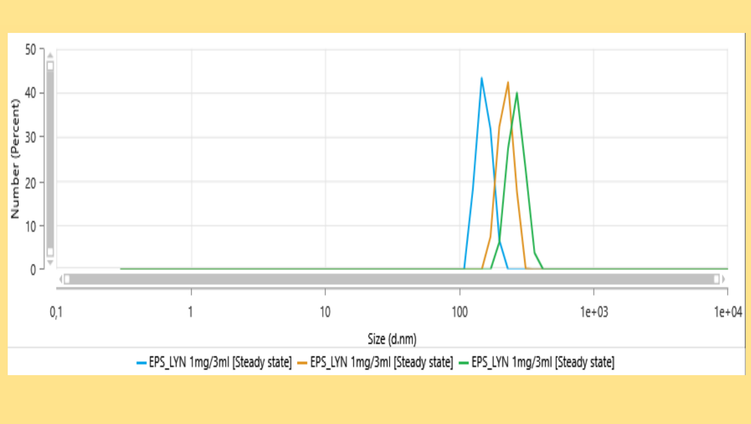


FTIR spectra of Lyngya sp., both endo- and esopolysaccharides. Each peaks is due to the movement of a specific functional group that has been excited by the infrared radiation.

λ (cm-1)	functional gr.
3200 – 3309	-OH
2860 – 2950	-CH
1720 – 1736	CH=O
1609 – 1650	-COOH
1540 – 1600	-NH
1360 – 1420	-S=O
1010 – 1060	-CO
804	- COS

From the DLS measurements , it appears the sample of endopolysaccharides (left) extracted from SAG 11-32b are homogenous with only one population. While the esopolysaccharides sample of *Lyngbya* (right) seem more heterogeneous, as the peaks do not overlap each other, than could be due to an uncompleted dissolution of the sample.





The tab shows the average content of carbohydrates and proteins content as percentages.

sample	% carb.	% prot.
CC 125	11±5	3±2
SAG 11-32b	12±2	9±3
Porph.	10±3	1.0±0.3
EPS_Porph	13±1	-
Lyngbya	23±10	2±1
EPS_Lyngbya	10±2	-

The tab shows the average quantity of the monosaccharides identified with the GC-MS analyses.

monosaccharides	%
glucose	31±3
mannose	39±3
rhamnose	30±3

Although a complete characterization of the extracts is still in progress, all the mixtures are mainly composed of carbohydrates, in the form of polysaccharides, and proteins. The UV-Vis absorbance spectra, together with FTIR results, confirm the presence of these two macromolecules. The peaks observed belong to the functional groups of monosaccharides chains (-OH, -CO, -COOH) and proteins ($-NH_2$). The amount of carbohydrates and protein were measured by means of colorimetric assay: UV-sulfuric acid method to quantify the content of total reducing sugar, while Bradford method for the protein. Gas-chromatography was performed on the sample, to recognise the type of monomers of the polysaccharides: it was observed the presence of glucose, mannose and rhamnose.

The CHNS elemental analysis allows to measure the amount of certain elements, by combustion of the sample (1000°C), followed by catalytic oxidation and reduction processes. Then the produced gases are separated in a gas-chromatographic column and analyzed with a thermal conductivity detector.

sample	N	C	H	S
Chlam. r.	8,7±0.1%	33,6±0.1%	5,4±0.1%	0±0.1%

Next steps

- characterization of polysaccharides' chains by means of GC-MS and HPLC
- identification of the protein content
- analysis of the gel structure and its physical properties
- application of the hydrogel on paper specimens, and study of its stability by means of cycle of artificial ageing.