

## BioMiMe

Biorefinery of Microalgae by Membrane technology for the production of bioactive ingredients

### SUMMARY

The main aim of the BioMiMe Democase was to understand and prove the effectiveness and potentialities of membrane technology to bio-refine spirulina microalgae while preserving the properties of bioactive ingredients and purity. To achieve this knowledge the project pursued the following general research objectives (GRO):

- Increase specific knowledge on the extraction of biocompounds from *Spirulina*;
- Identify the best sustainable technologies for the extraction;

### THE CONTEXT

There is an increasing demand for drugs produced with active ingredients of natural origin, known as phytochemicals compounds (PC). The EU faces a shortage of active ingredients due to the COVID-19 pandemic and the geopolitical situation. These factors are pushing European companies to diversify imports and/or bring production back to Europe.

ISGREEN is a small enterprise active in developing bioderived products based on *Spirulina microalgae* cultivated in photobioreactors under controlled conditions (absence of contamination). Microalgae *Spirulina* is a source of active ingredients, among them the most important in terms of quantity is phycocyanin. The Global *Spirulina* Market was valued at \$393,6 million in 2019 and is projected to reach over \$900 million by 2027.

Extraction of phycocyanin, purification, and preservation of its bioactivity is still a challenge.

Within BioMiMe, CNR-ITM in close cooperation with ISGREEN, advanced knowledge on how to overcome current challenges in the extraction of phycocyanin, purification, and preservation of its bioactivity by using membrane technology.

#### Microalgae Spirulina: a source of active ingredients



Spirulina is composed of numerous **bioactive ingredients**, among them the most important in terms of quantity is **phycocyanin**

Spirulina biomass has been certified as Novel Food in Europe, and it is used as a functional food

The Global *Spirulina* Market was valued at **\$393,6 million in 2019** and is projected to reach over **\$900 million by 2027**

Properties:  
Anti-inflammatory  
Immunostimulant  
Antioxidant  
Antiradical

Food colorant

It is the only natural **blue dye** allowed in Europe

**Extraction of phycocyanin, purification, and preservation of its bioactivity is still a challenge**



## THE CHALLENGE

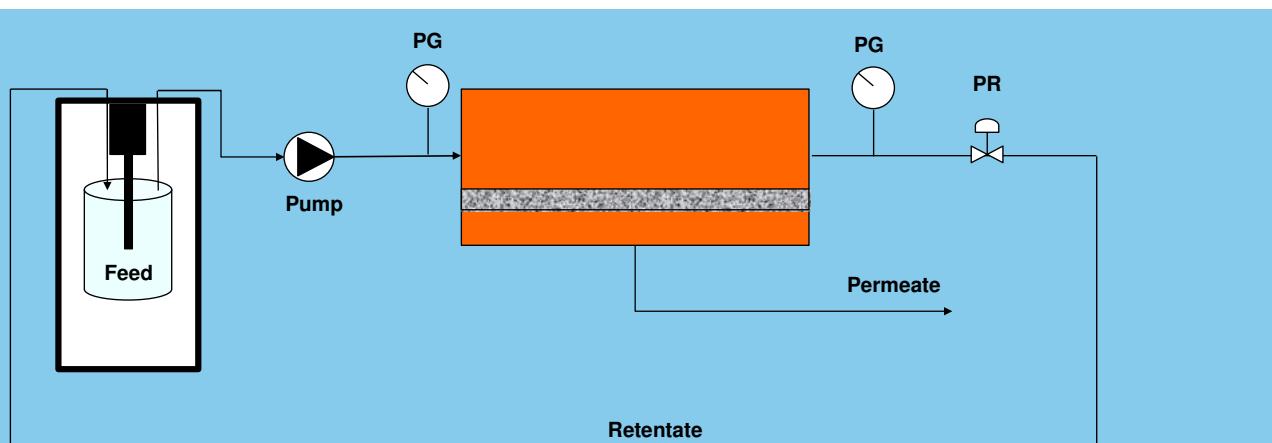
Extraction of phycocyanin that preserves the bioactivity is still challenging.

BioMMe aims to overcome the current challenges in the extraction of phycocyanin, purification, and preservation of its bioactivity by using membrane technology

### *Observation at the optical microscope*



The effect of ultrasound treatment time on cell lysis



Schematic of MF or UF plant

## THE RESULTS

The following results were obtained:

- Identification of the pre-treatment to clarify the cell lysate suspension
- Identification of membrane material, pore size, and process conditions for MF and UF to purify and concentrate phycocyanin and maintain its bioactivity
- Achievement of 5 fractions:
  - 1) Spirulina cells harvested and purified from the broth (the broth can be reused in the photobioreactor)
  - 2) MF retentate with cell fragments, 50 ( $\pm$  5)% of phycocyanin (strategy 1) and 25 ( $\pm$  5)% of phycocyanin (strategy 2)
  - 3) MF permeate with phycocyanin and chlorophylls A and B
  - 4) UF retentate with food-grade phycocyanin
  - 5) UF permeate with purified water (that can be reused in the process/ photobioreactor).

The Key performance indicators were achieved:

- A pressure-normalized flux higher than  $50 \text{ L m}^{-2} \text{ h}^{-1} \text{ bar}^{-1}$  using permeate of MF as feed of UF membranes
- A rejection of phycocyanin through UF membranes higher than 90%
- A fouling index for MF membranes of 0.1.

Overall, the results and KPI are very promising and well beyond the state-of-the-art.

## CONCLUSION

Through membrane processes, pure phycocyanin was extracted from Spirulina Algae:

- Purity:  $A_{615}/A_{280} = 1.4$
- Suitable for food grade
- High-protein conc. solution



- **Brilliant blue appearance**



- **Fluorescence under light**

## TECHNIQUES USED

- Ultrasound to break the spirulina cells
- Microfiltration to clarify the cell lysate suspension and collect phycocyanin in the MF permeate
- Ultrafiltration to concentrate the purified phycocyanin
- Membrane emulsification
- Enzyme immobilization to functionalize membrane surface
- Water contact angle meter to analyze membrane surface hydrophilicity
- UV-VIS Spectrophotometer to analyse proteins
- Circular dichroism (CD) spectrometer to analyze protein configuration
- Resonance light scattering (RLS) to analyze protein aggregation

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