

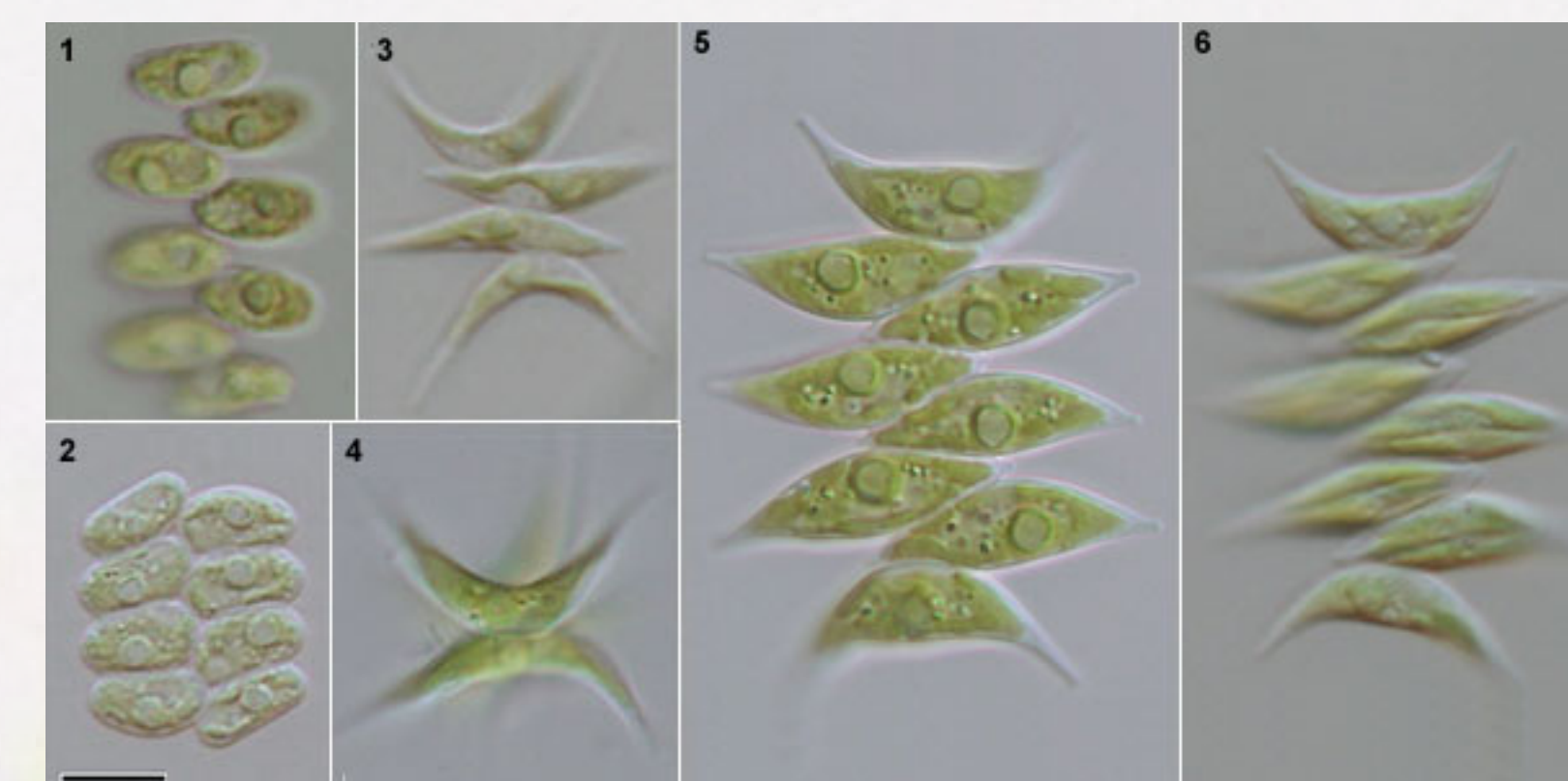
PRESURIZED LIQUID EXTRACTION AND SUPERCRITICAL FLUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM THE MICROALGA *Scenedesmus obliquus*

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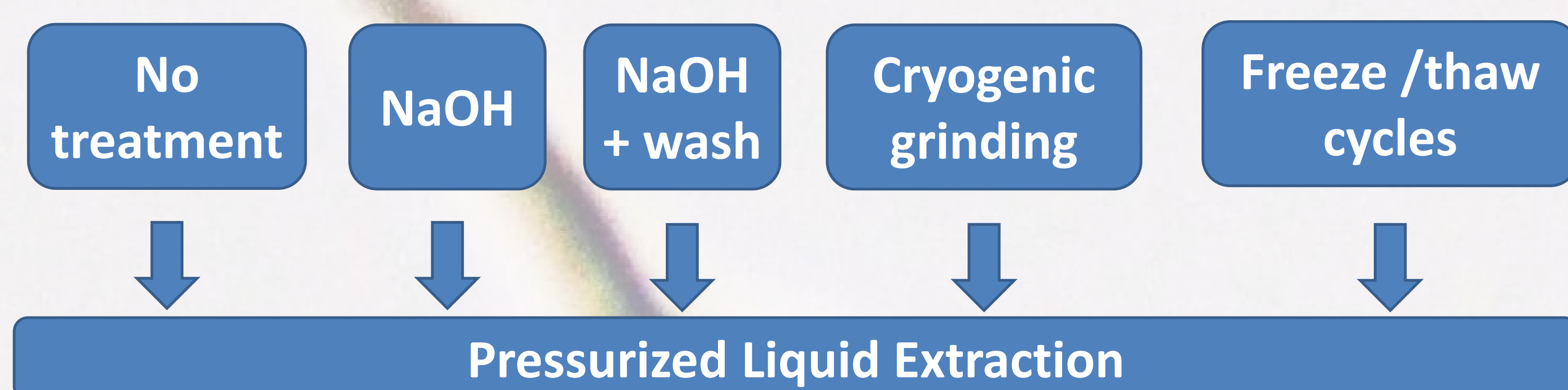
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Scenedesmus obliquus MICROALGA

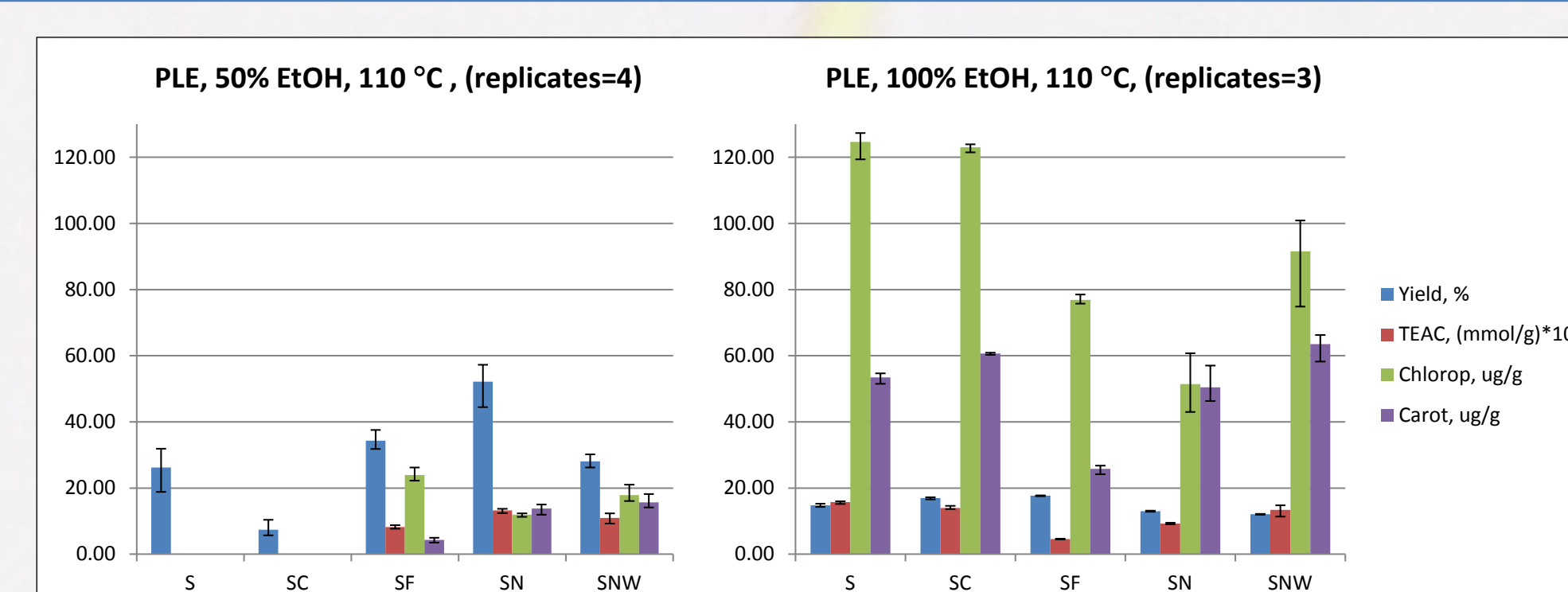
- *Scenedesmus obliquus* is a green microalga (Chlorophyceae) that grows in fresh water. It has been found in rivers from Andorra, Málaga and Balearic Islands among others [1]. Nowadays it's well known as a green microalga but originally it was classified as a diatom by Kützing [2].
- This microalga has a fast growth rate. It contains approximately 55% of proteins, 14% carbohydrates and 13% of lipids. It's appreciated by its content in specialty oils (18:3 omega-3), proteins, pigments, carotenoids (lutein), specialty carbohydrates and sterols among others
- Despite it's very well known in aquaculture and in highly valued for biodiesel production, due the important concentration of bioactive compounds, it has a very strong cell wall that makes the extractions more difficult.



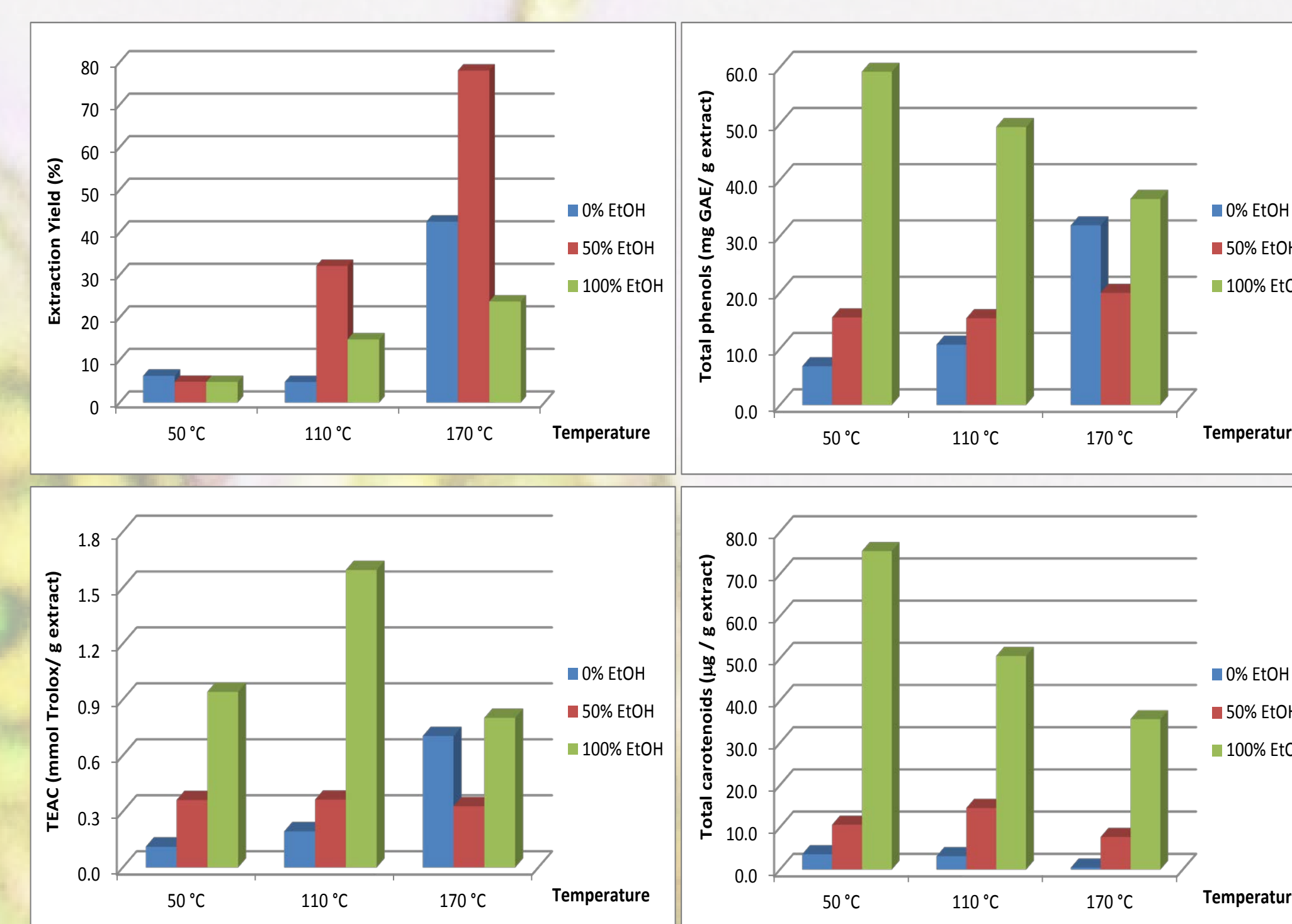
PRETREATMENT: CELL WALL DISRUPTION



Due to the strong cell wall of *S. obliquus*, four different pretreatments were studied. The highest yield was obtained after basic hydrolysis without removing NaOH residues, but the yield decreased when NaOH residues were removed by washing with water until neutral pH, and the obtained value was similar to the yield obtained by Pressurized Liquid Extraction (PLE) extraction of untreated *S. obliquus*. The washing step improved carotenoids and chlorophylls detection. For PLE, an **experimental design (3-level factorial design, 3²)** was carried out for extraction using considering: solvent (Ethanol:water 0 to 100% ethanol) and Temperature (50 to 170 °C). Best results were obtained at 50 °C and 100% ethanol as extracting solvent.



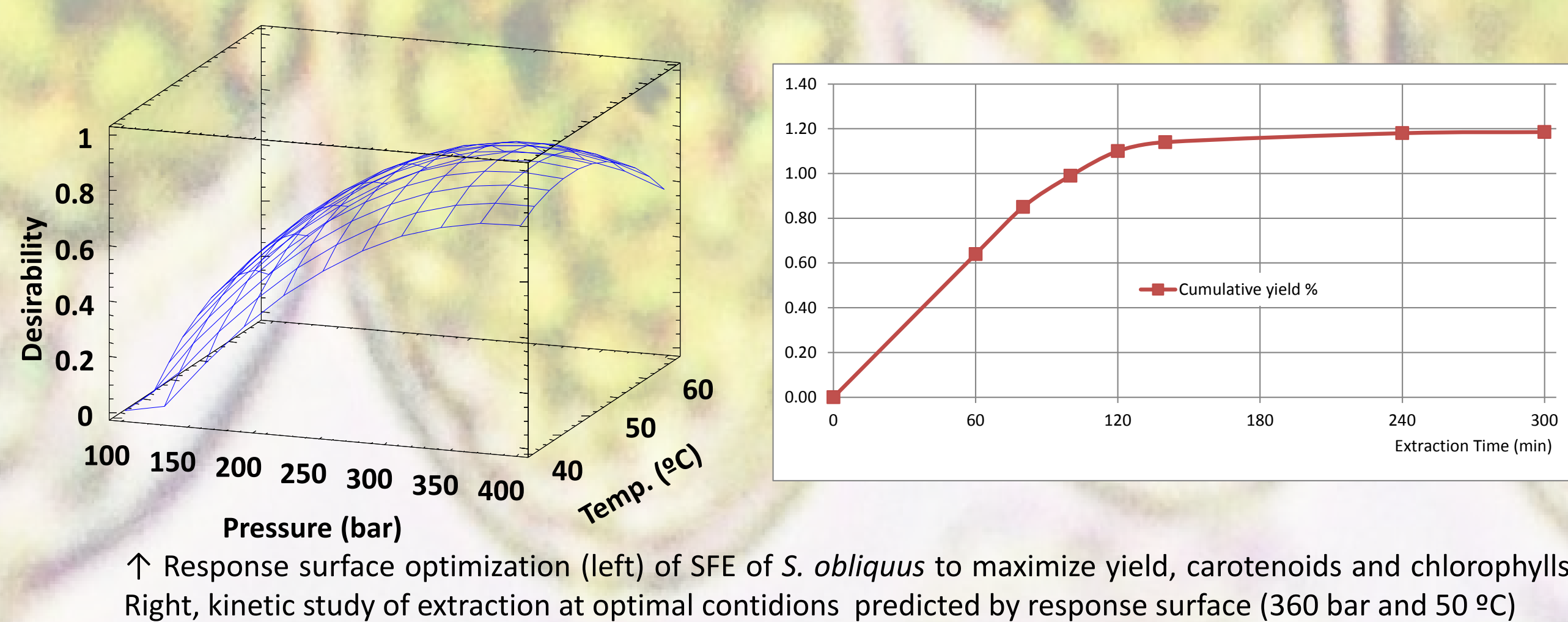
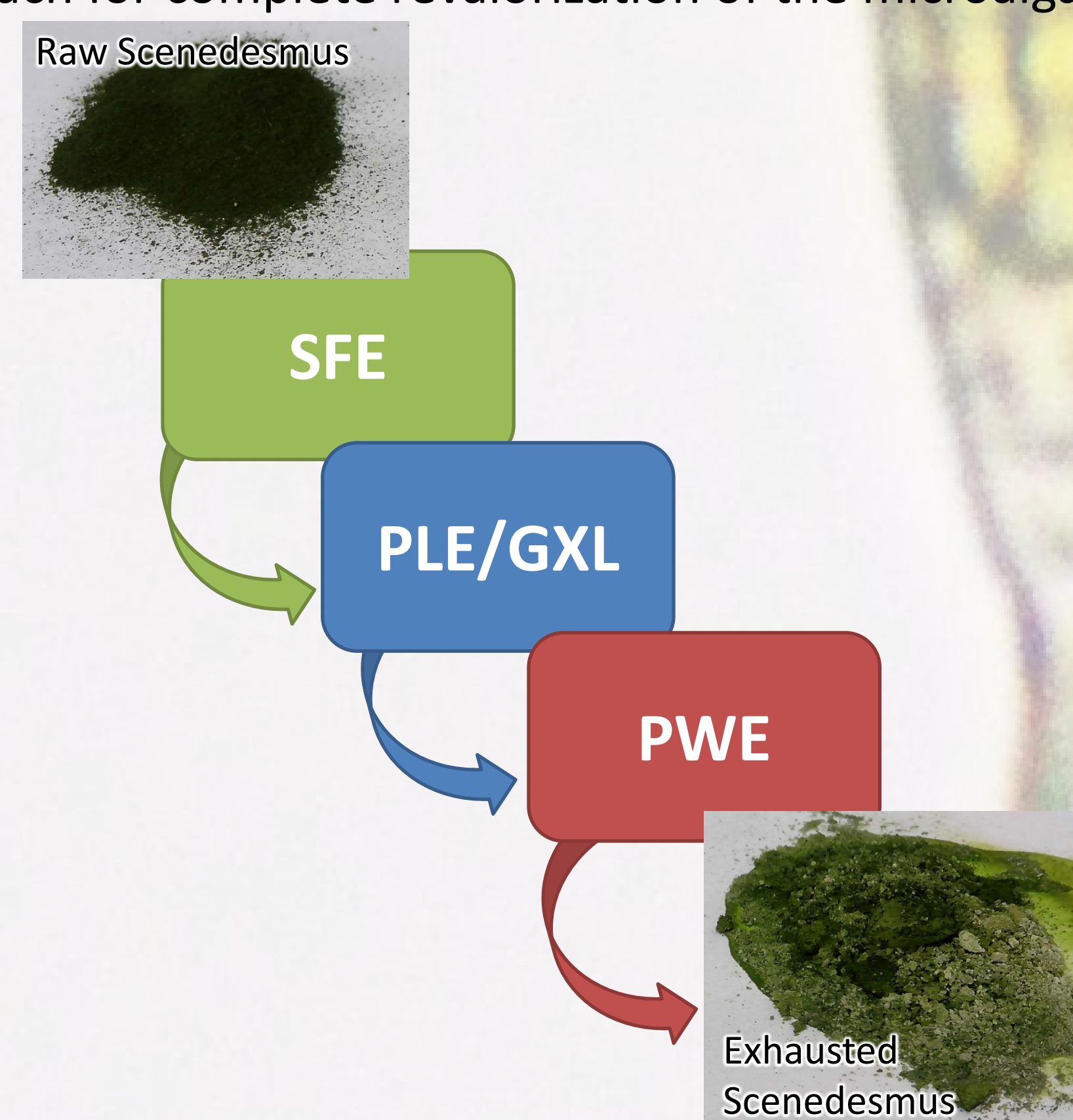
← Extraction yield (%), 10-fold antioxidant activity (TEAC, mmol/g), total chlorophylls (µg/kg) and total carotenoids (mg/kg) of ethanol and ethanol/water (50:50) PLE extracts of *S. obliquus*, after different treatments. S= no treatment; SC= cryomill; SF= freeze-thaw; SN= NaOH; SNW= NaOH+wash.



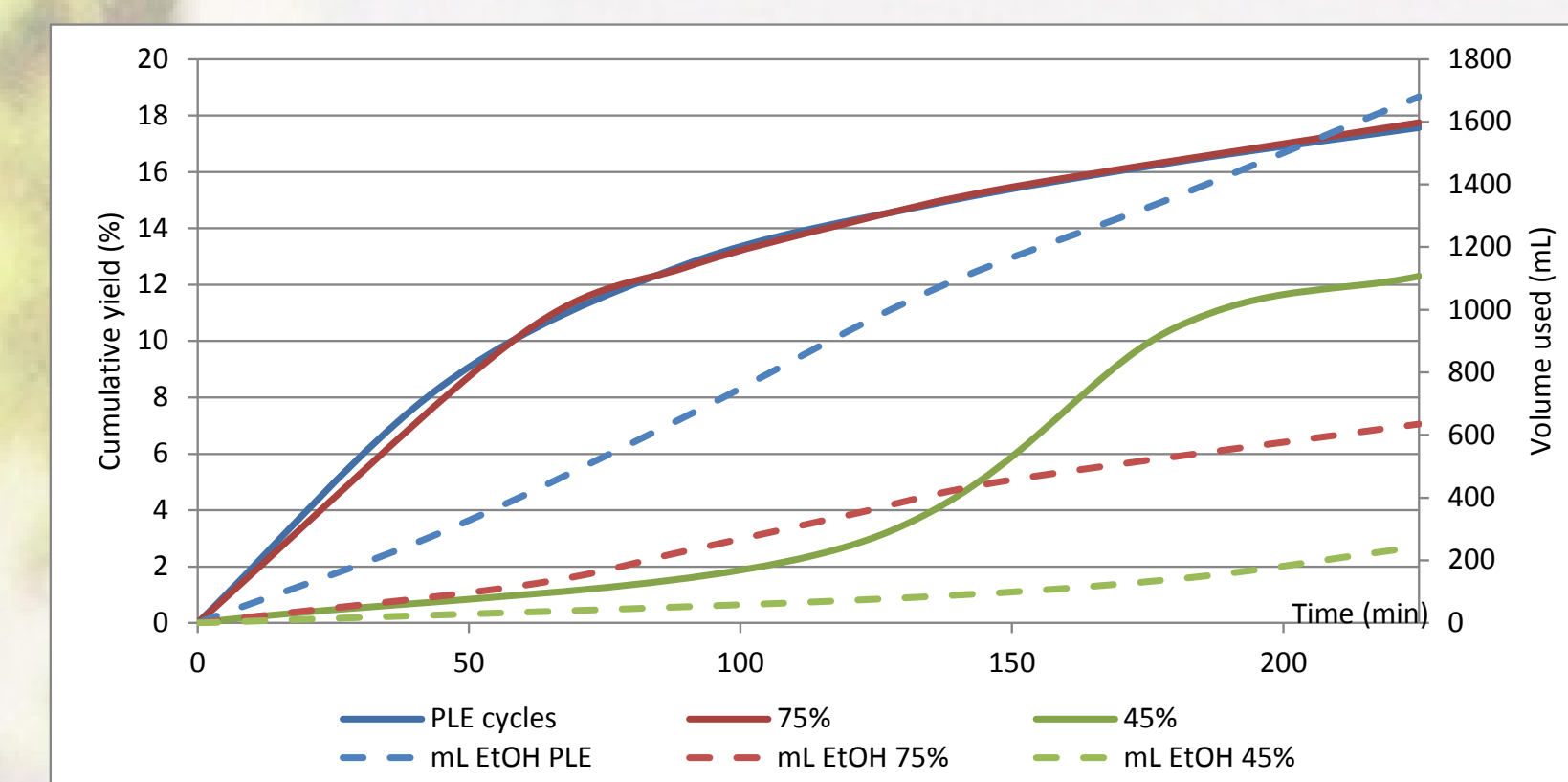
← Extraction yield, total phenols, total carotenoids and antioxidant activity (TEAC) of the *S. obliquus* PLE extracts produced at different extraction temperatures with the indicated solvents.

BIO-REFINERY PROCESS

Following the results showed above and previous results obtained for another microalga [3] a sequential steps extraction process has been set up in order to obtain the maximum different extracts from *Scenedesmus obliquus* microalga. The green downstream platform developed in this study produced different extracts with potential for application in the food, pharmaceutical and cosmetic industries. Therefore, a good approach for complete revalorization of the microalgae biomass is proposed, by using processes complying with the Green Chemistry principles.



↑ Response surface optimization (left) of SFE of *S. obliquus* to maximize yield, carotenoids and chlorophylls. Right, kinetic study of extraction at optimal conditions predicted by response surface (360 bar and 50 °C)



↑ Kinetic study of the second step showing yield vs time in left axis and volume used in right axis (dashed lines) of the three conditions tested.

Extractions were performed in three sequential steps considering the residue of the previous extraction step as the raw material for extraction. After optimization, the elected extraction conditions for the *Scenedesmus* bio-refinery process are:

1. ScCO₂ 360 bar and 50 °C (low polarity). **Supercritical Fluid Extraction, SFE**
2. 75:25 (Ethanol:ScCO₂), 50 °C, 60 bar (mild polarity). **Gas Expanded Liquid extraction, GXL**
3. Water at 70 bar, 50 °C (high polarity). **Pressurized Water Extraction, PWE**

CONCLUSIONS

A downstream processing platform is described for the first time to extract bioactive compounds from the microalga *Scenedesmus obliquus* using GRAS –generally recognized as safe– solvents and pressurized technologies.

References

1. Guiry, M.D. & Guiry, G.M. 2015. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>
2. Kützing, F.T. Synopsis diatomearum oder Versuch einer systematischen Zusammenstellung der Diatomeen. Linnaea (1834 '1833')8: 529-620, pls XIII-XIX
3. Gilbert-López, B., et al "Downstream processing of Isochrysis galbana: A step towards microalgal biorefinery" Green Chemistry, 2015, 17 (9), 4599 - 4609

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