

Improved technique for lipids extraction Lipids analysis by GC-MS and Flow Cytometer

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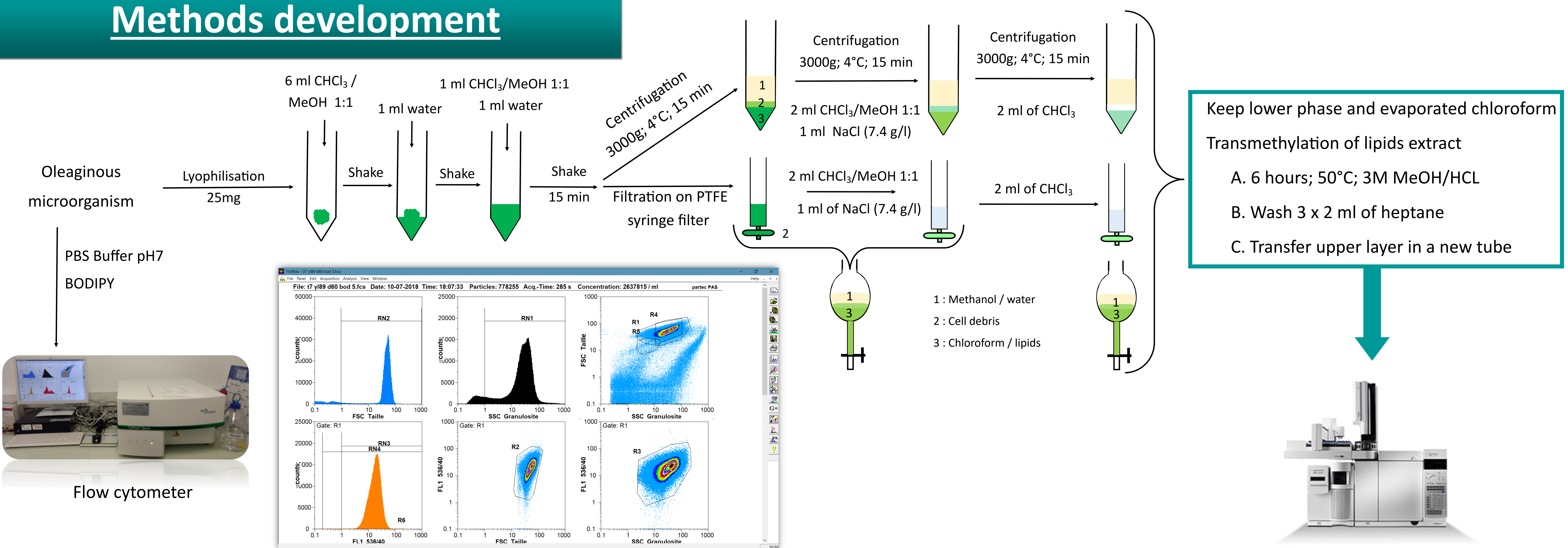
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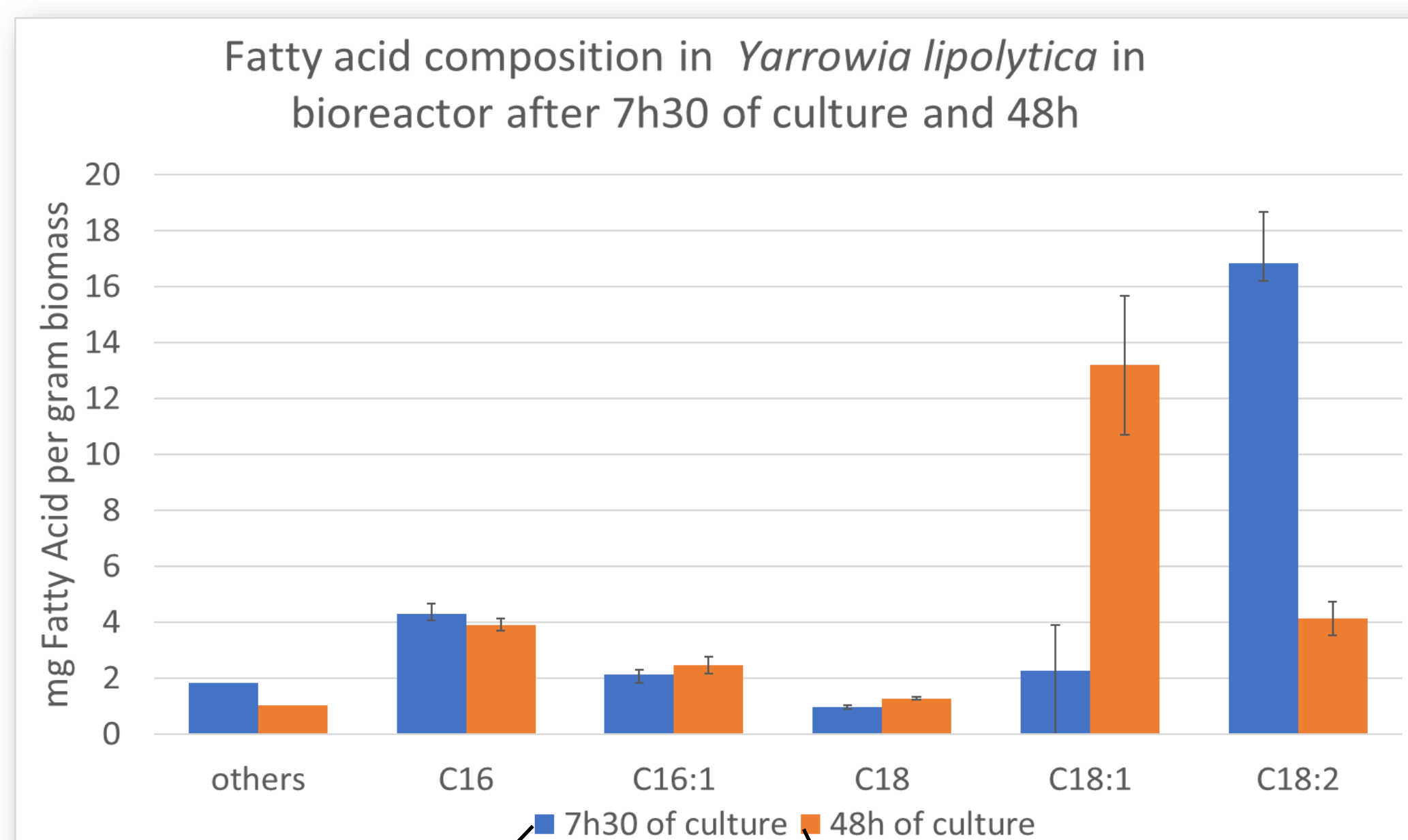
Introduction

The concept of biorefinery aims to accommodate economic, demographic growth with sustainable development. The objective of Alpo project is to valorize microalgae lipids to bioplastics. In this context of biorefinery all of "waste" must be valorized. The product obtained after lipids extraction from microalgae is mainly composed of sugars, that can be transformed through fermentation by oleaginous yeasts to high added value unusual lipids. Analysing lipids from microorganisms like yeasts or microalgae is quite challenging as the method must allow accessing lipids within the various cell structures (cell wall, vacuole). Here we developed a single method based on Bligh and Dyer method¹ for lipid extraction adapted to yeasts and microalgae. We also compared the lipids profile by GC-MS. In parallel, we are currently developing a non destructive method to quantify lipids during fermentation using flow cytometry.

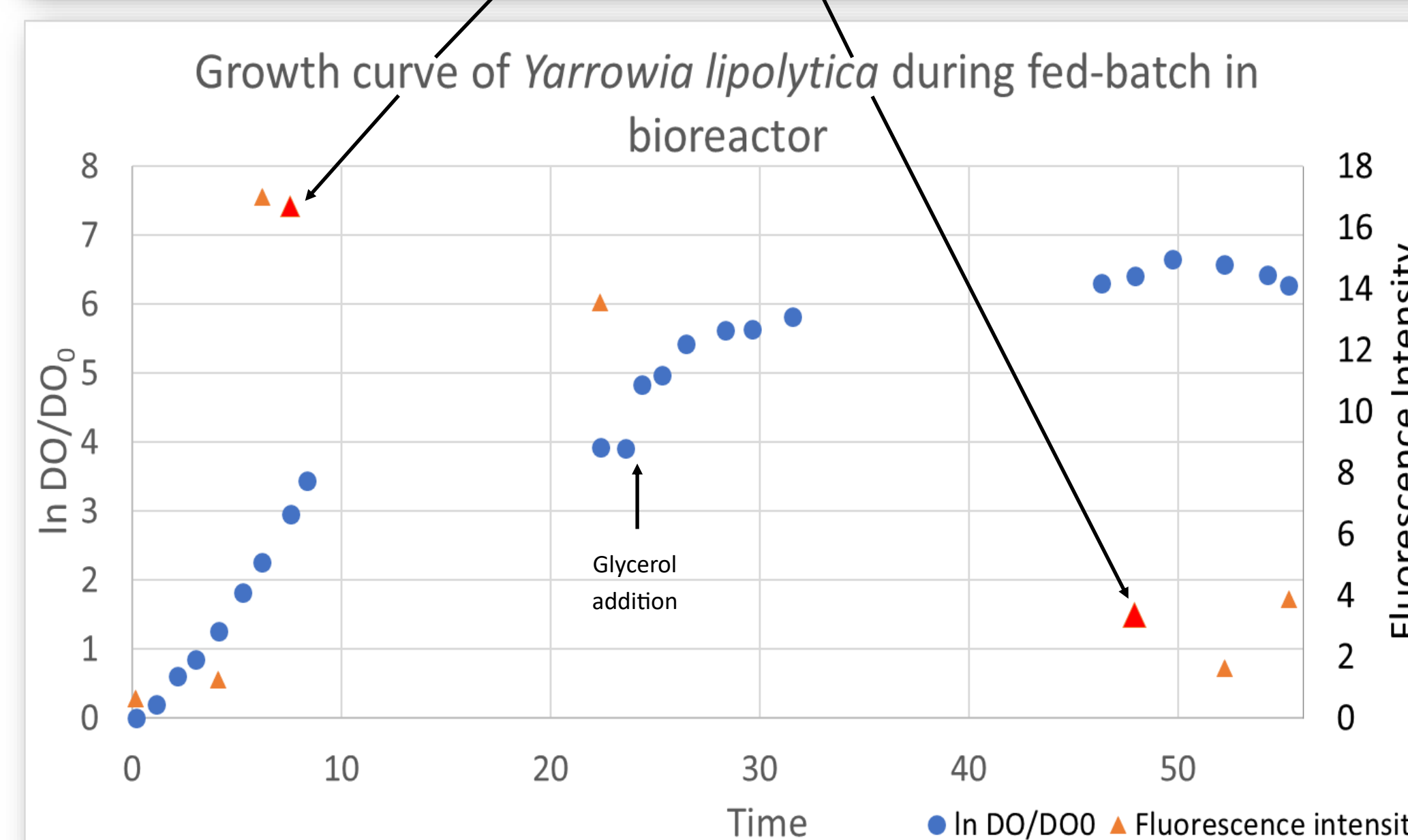
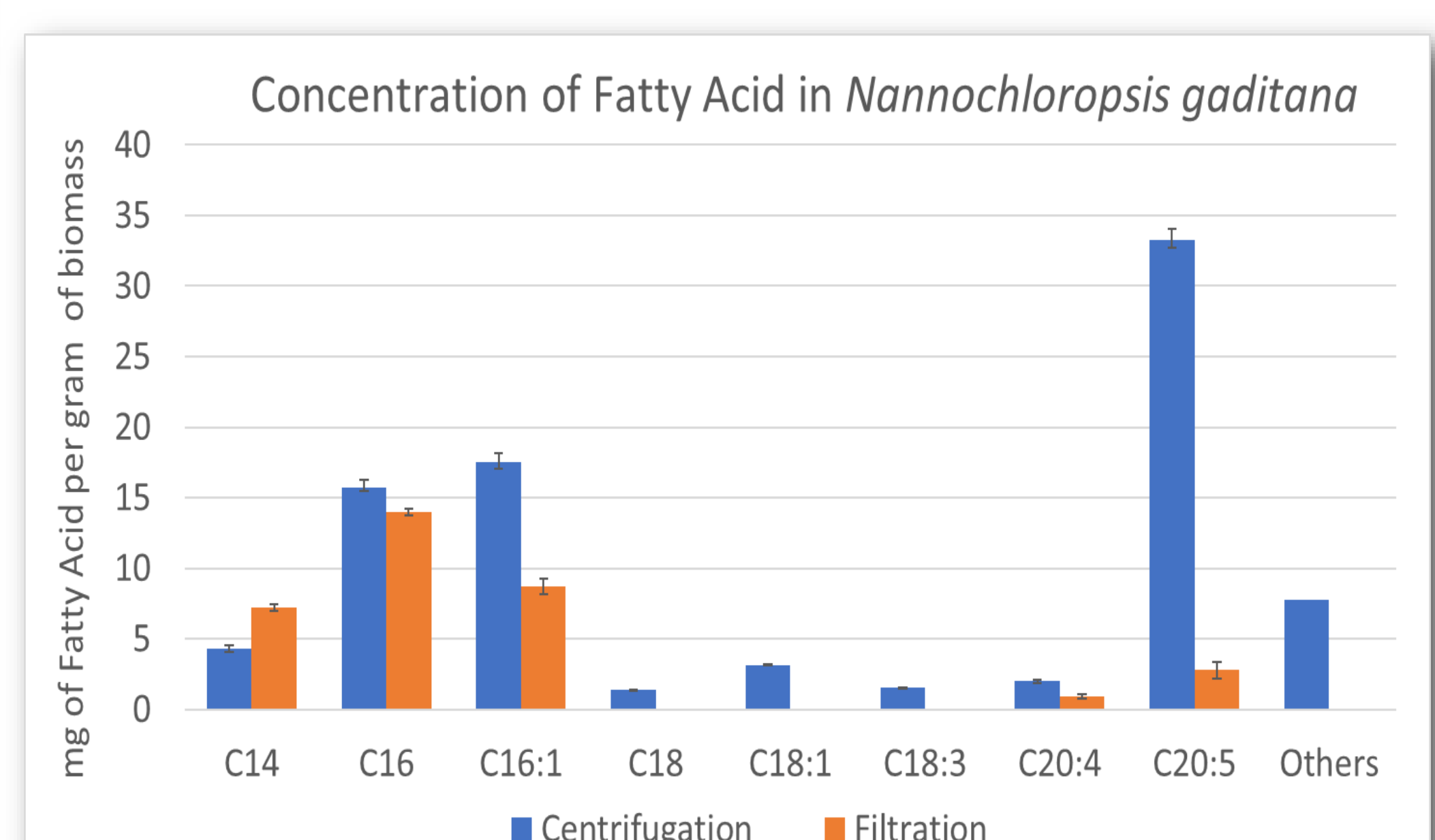
Methods development



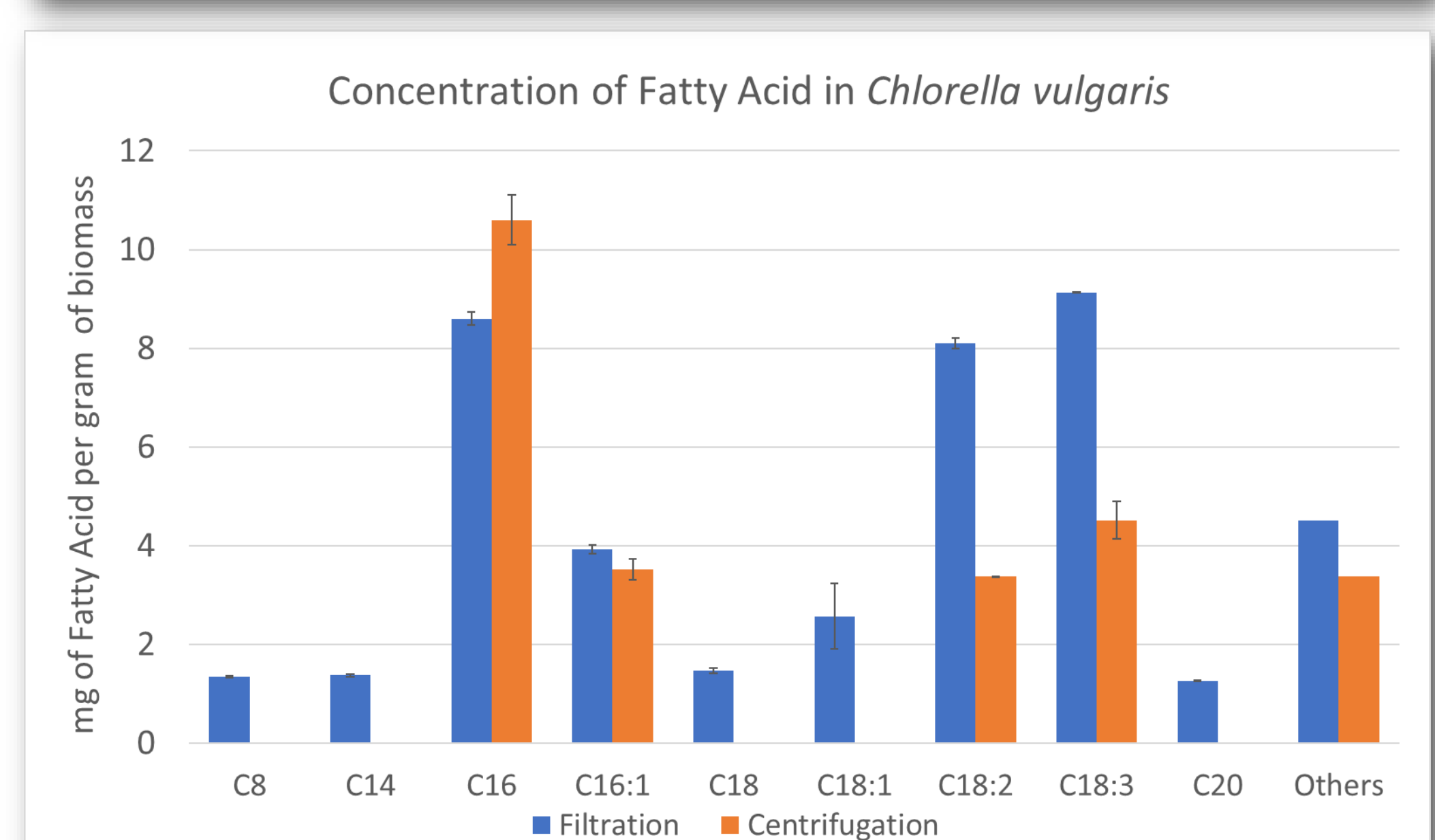
Results and Conclusions



- Variation of fluorescence intensity during fermentation
 - Huge intensity at the end of exponential phase
 - Variation of fatty acid composition in *Yarrowia lipolytica* during growth
- ⇒ Fluorescence intensity and fatty acids are linked
- ⇒ Maybe a better affinity of Bodipy for unsaturated fatty acids could explain the difference in intensity between 7h30 and 48h of culture



- Centrifugation protocol works with the same efficiency as filtration protocol on *Chlorella vulgaris* and *Nannochloropsis gaditana*
- ⇒ Extraction of fatty acid is better with centrifugation
- ◇ More fatty acid diversity
 - ◇ Less measurement inaccuracy of fatty acid



Perspective

In this project, microalgae is the raw material of fermentation. The aim is to find microorganisms able to produce unusual fatty acids from microalgae by-product. A method using flow cytometry was developed. This method allows following instantly lipids production during fermentation and helps us selecting the best yeast strain for lipids production.

Reference

1. Bligh EG, Dyer WJ. A RAPID METHOD OF TOTAL LIPID EXTRACTION AND PURIFICATION. *Can J Biochem Physiol.* 1959;37(8):911-917. doi:10.1139/o59-099.

Acknowledgement

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