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Release of free fatty acids in *Chlorella* biomass during post-harvesting steps

Tiana Reynald¹, Emmanuelle Lautié¹, Nathalie Barouh^{2,3}, Victoria Chuat⁴, Valérie Gagnaire⁴, Florence Valence⁴, Claire Bourliou-Lacanal¹, Maeva Subileau^{1*}

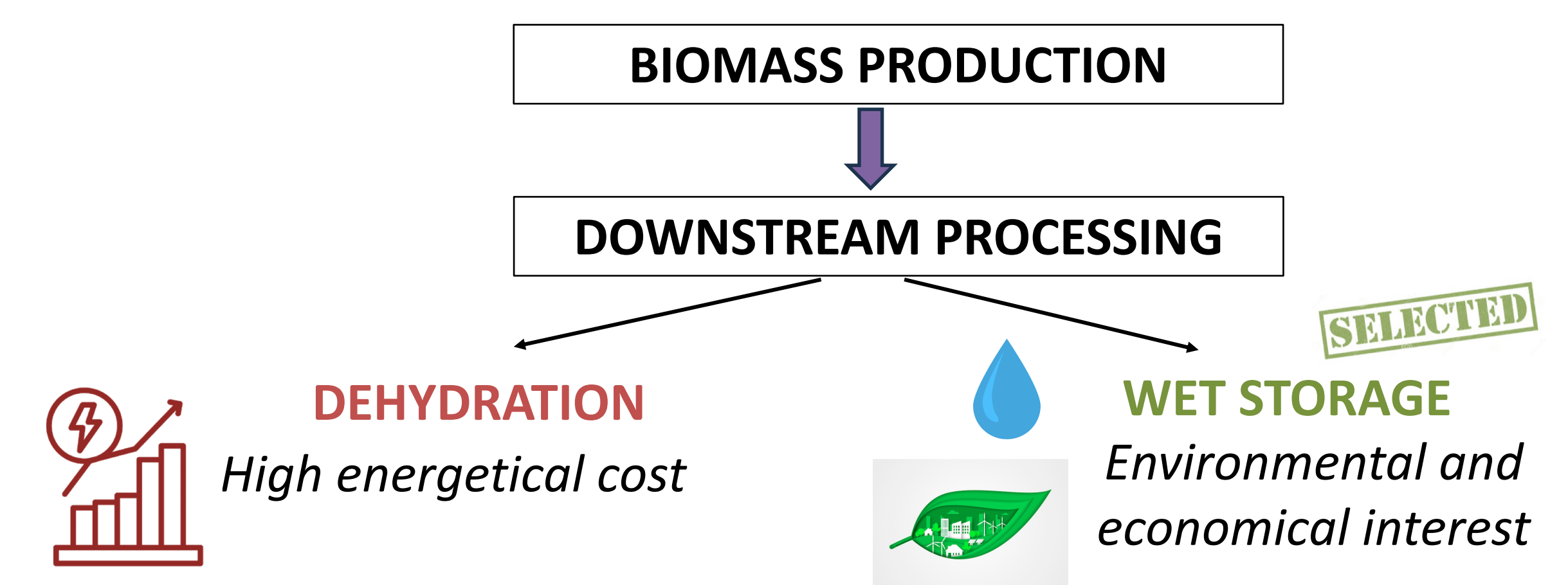
*maeva.subileau@supagro.fr

¹UMR IATE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France, ²CIRAD, UMR QUALISUD, F34398 Montpellier, France, ³QUALISUD, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, Université de La Réunion, Montpellier, France, ⁴UMR STLO, INRAE, Institut Agro, F35000, Rennes, France

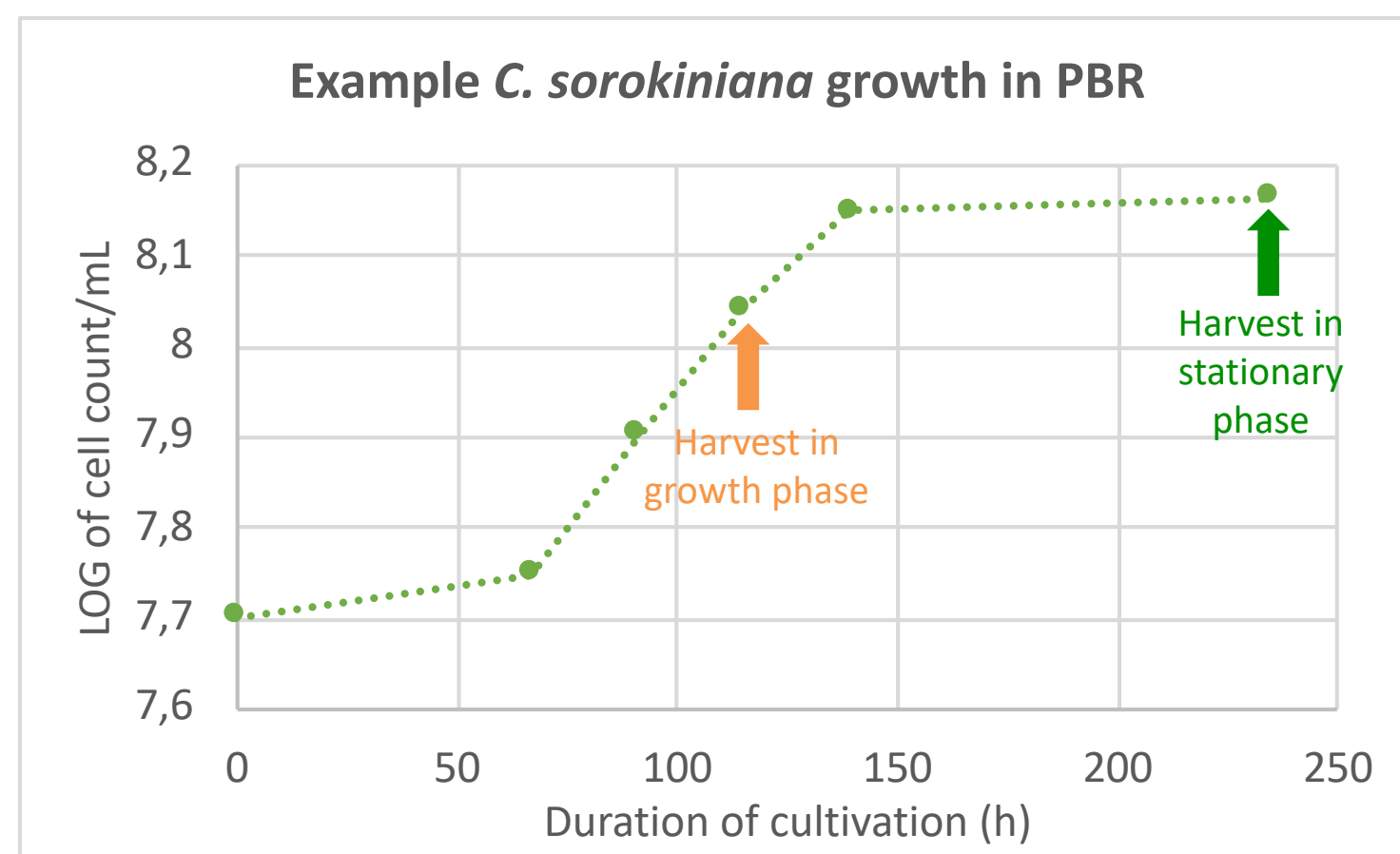
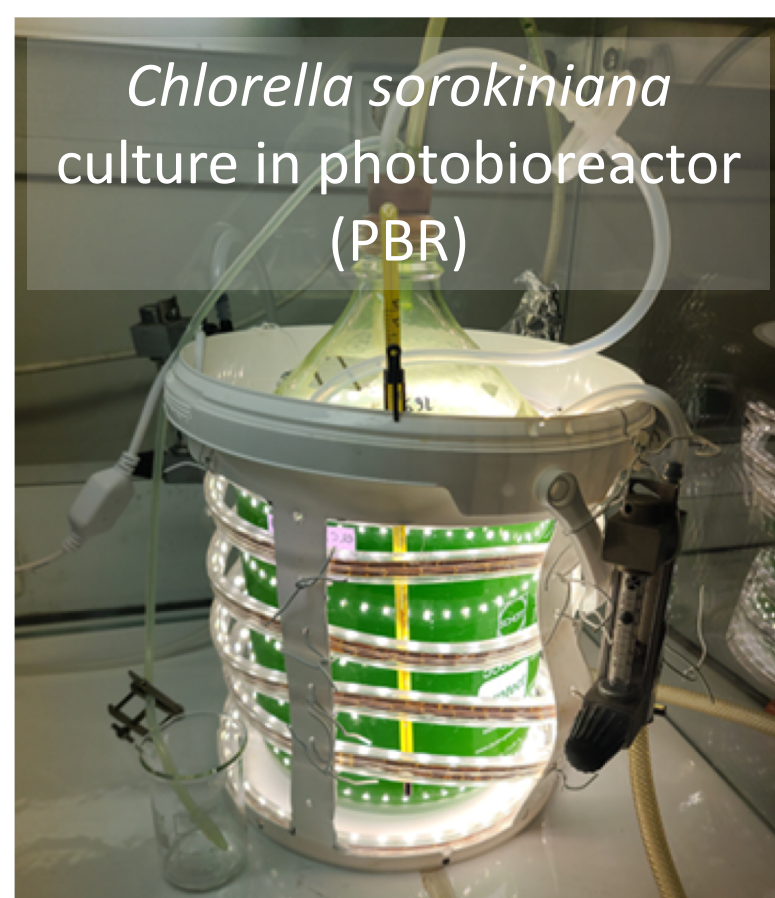
Within the European legislative framework, microalgae market for human consumption is dominated by products originating from "Spirulina" and "Chlorella" whole biomass. In this context, *Chlorella* is marketed for its **health-promoting functional lipids**, among other properties. Still, the **quality of the lipids** contained in *Chlorella* biomass can vary significantly (Barouh et al., 2024). Particularly, **the level of free fatty acids (FFA) in the product can be detrimental** to flavour and nutritional properties. Indeed, FFA can be naturally released from glycerolipids by lipases present in microalgae cells (Balduyck et al. 2017, 2016). To circumvent lipolysis, microalgae are mostly sold under dried form, but wet downstream processing has environmental and economical interests. Therefore, challenges need to be overcome in terms of understanding and determining the optimal conditions to control of fatty acid release.

→ **Endogenous lipolysis during wet storage, its reaction kinetics and the factors that can allow limiting FFA release need to be better characterized in *Chlorella*.**

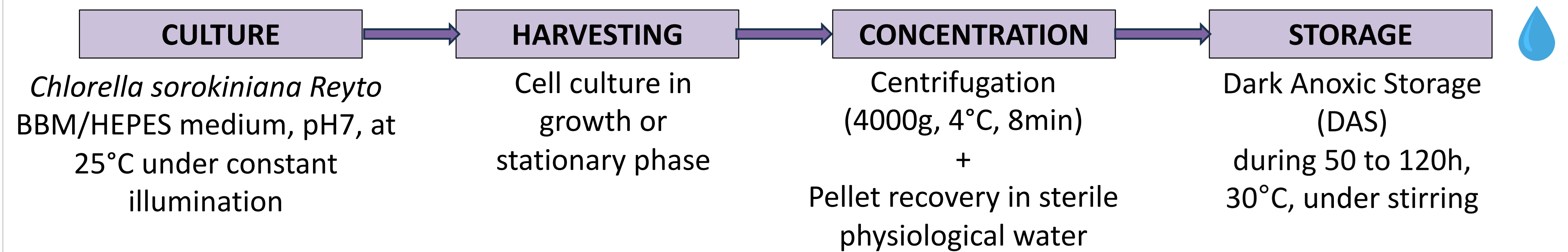
Whole cell microalgae processing steps



Cultivation and wet storage conditions



Chlorella culture, concentration and wet storage parameters

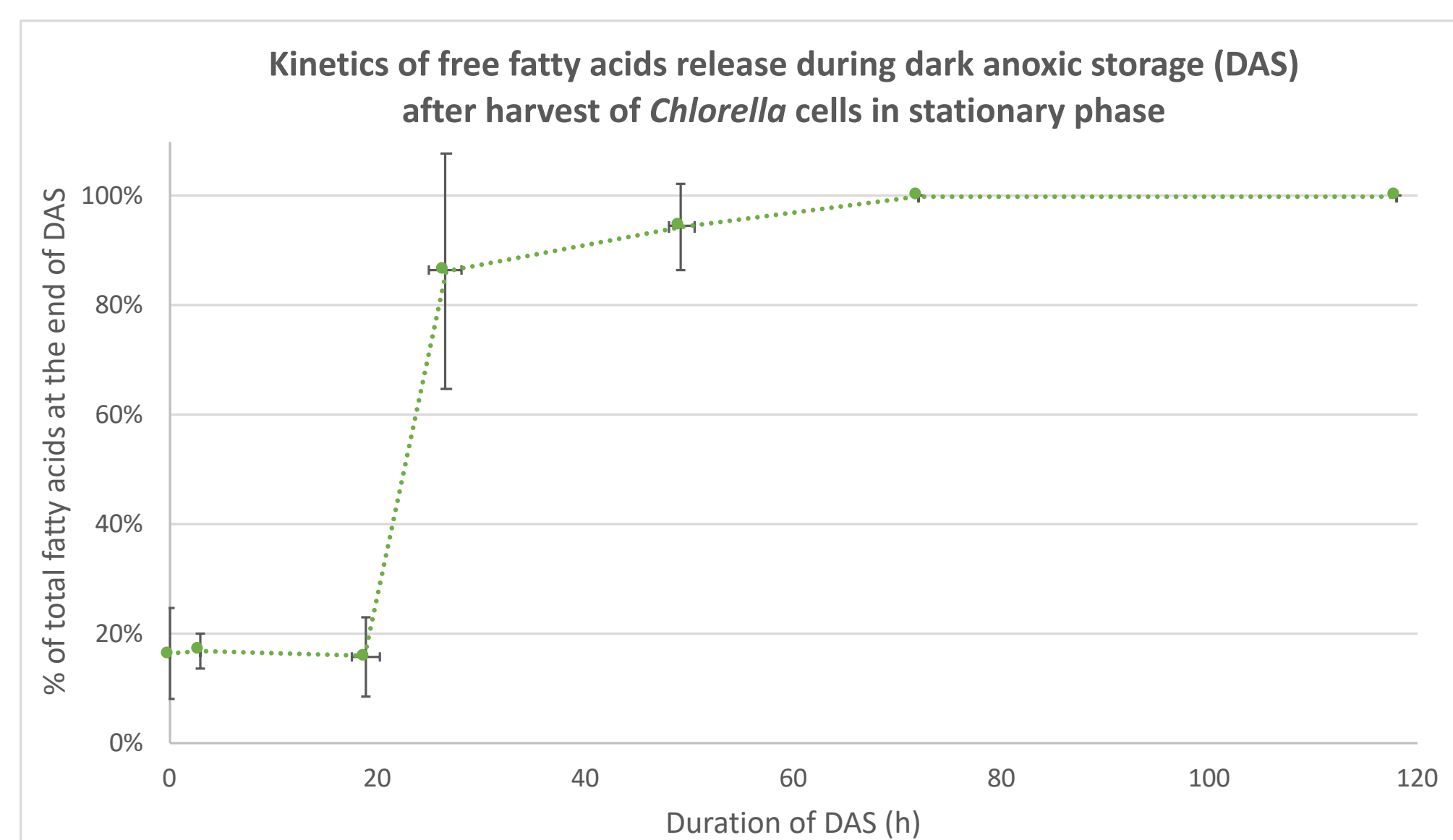


RESULTS : LIPOLYSIS DURING DARK ANOXIC STORAGE (DAS) was evaluated through the hydrolysis of fatty acids and the free fatty acids released

Levels of FFA were assessed after liquid/liquid extraction with hexane, derivatization with *N-Methyl-N-trimethylsilyl-trifluoroacetamide* and quantification by GC-FID

(1) Kinetics of lipolysis during DAS

Time of harvest	Stationary phase
Duration of <i>C. sorokiniana</i> cultivation in PBR before harvest	280 h
Cells concentration at time of harvest	1.67 g/L
Cells concentration during storage (x40)	65.7 g/L



Lipolysis happened in two distinct stages during DAS

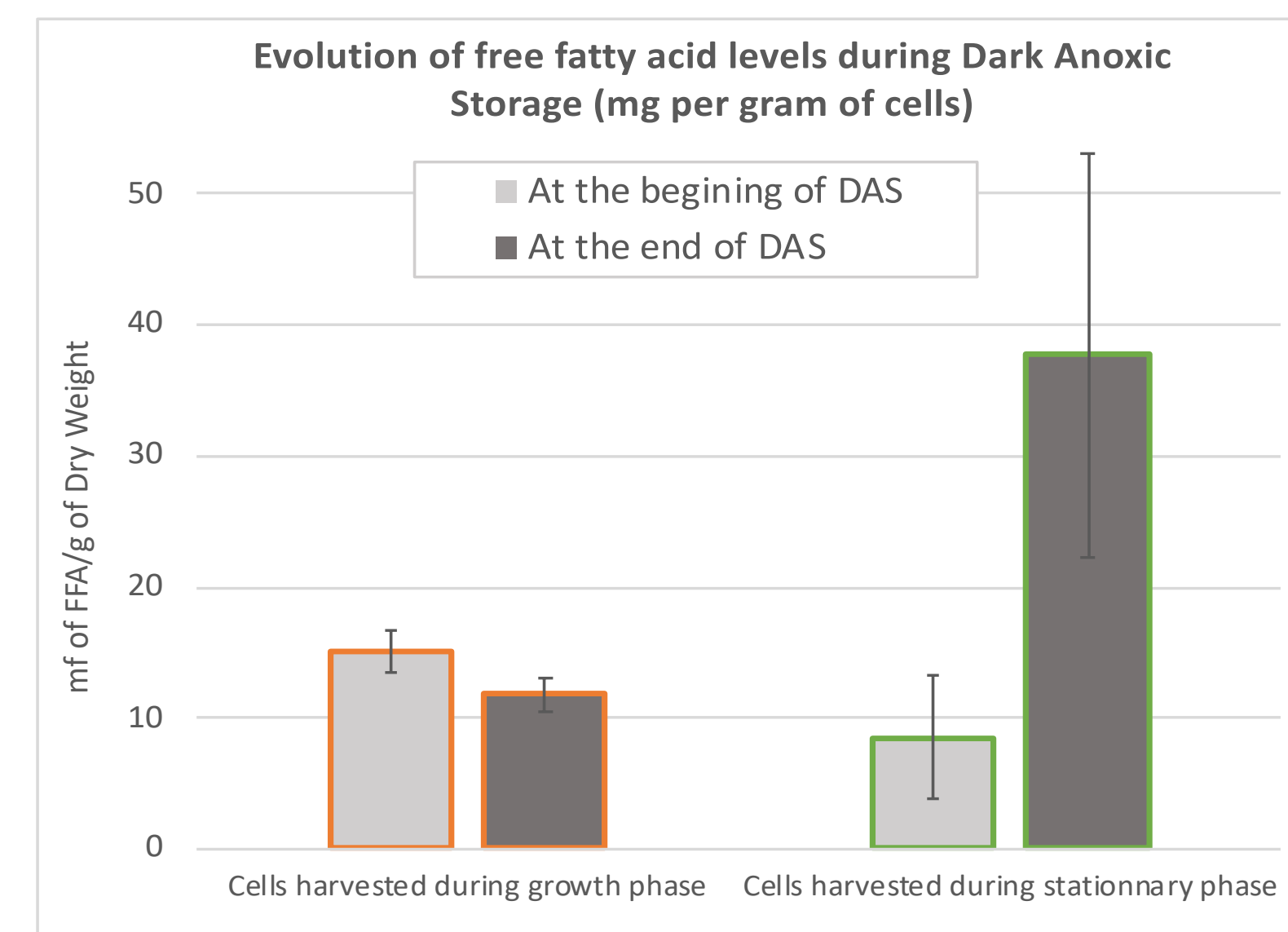
→ At the beginning of DAS, the level of FFA was about 15% of the final free fatty acids level obtained at the end of the DAS: considering that FFA level is very low in viable cells, a first stage of lipolysis must have occurred when cells were harvested, just after the concentration step. This initial level of FFA remained stable for 18 to 20h during DAS.

→ A second important and fast step of lipolysis was then observed: the level of FFA increased significantly after 18 hours of DAS, with a brutal rise, suggesting an important degradation of the cell composition. FFA reached, after 50 hours, a stable and maximum level 3 times higher than just after concentration.

(2) Effect of the harvesting time: cells in growth phase vs stationary phase

Time of harvest	Growth phase	Stationary phase
Duration of <i>C. sorokiniana</i> cultivation in PBR before harvest	115 h	280 h
Cells concentration at time of harvest	0.42 g/L	1.67 g/L
Cells concentration during storage (x40)	16.8 g/L	65.7 g/L

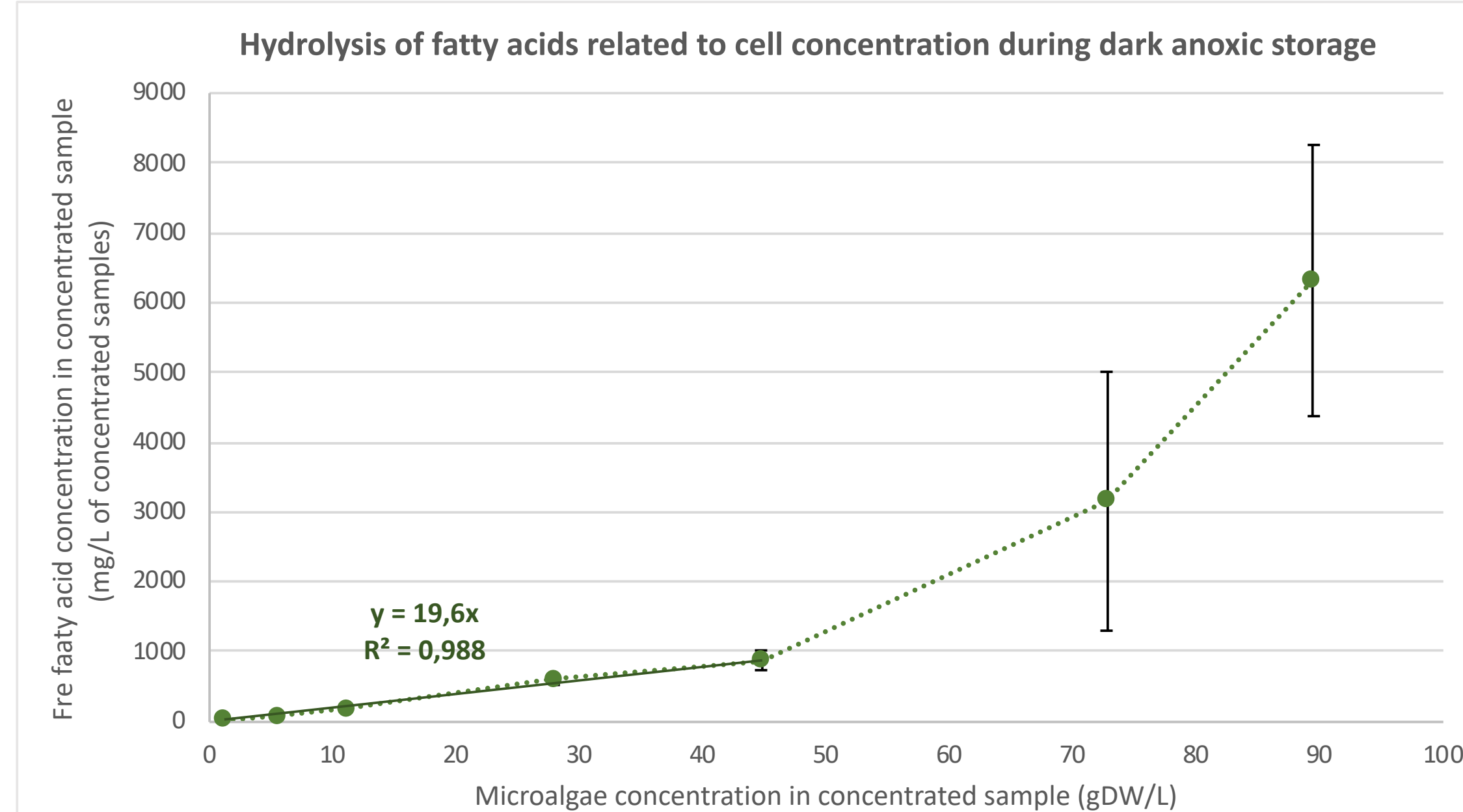
Microalgae cells harvested in growth phase displayed limited lipolysis during DAS



→ In cells harvested during growth phase, the level of free fatty acids did not evolve in the course of DAS. It remained stable at a value comparable to that obtained at the beginning of DAS when cells were harvested in stationary phase, *i.e.* the level of FFA corresponding to the first stage in lipolysis kinetics (§1). This observation could also be related to cell concentration (§3).

(3) Effect of the cell concentration factor on FFA release after 72h of DAS

Time of harvest	Stationary phase
Duration of <i>C. sorokiniana</i> cultivation in PBR before harvest	350 h
Cells concentration at time of harvest	1.12 g/L
Cells concentration during storage (x1 to x80)	1.1 to 89.5 g/L



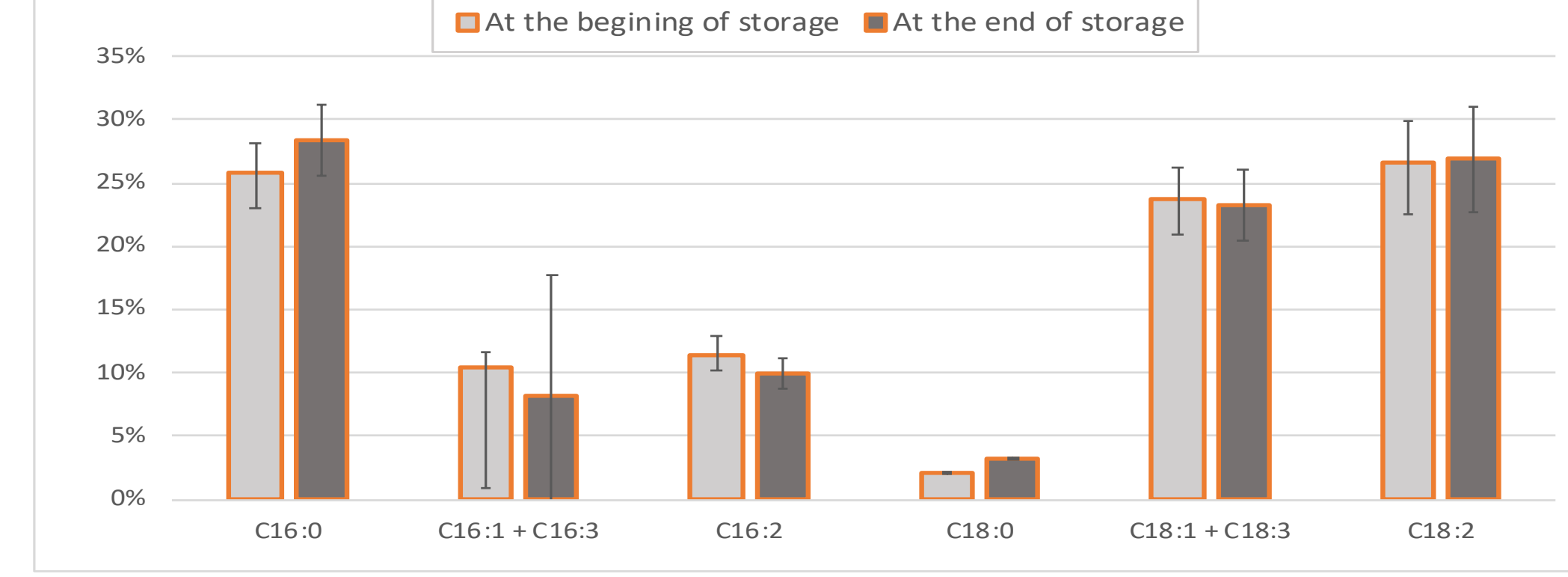
Lipolysis was more severe at higher cell densities as observed for in *Chlamydomonas* during DAS (Nami et al, 2022)

→ Below a cell density of 45 gDW/L, a linear correlation was observed between cell concentration and the maximum FFA level achieved at the end of DAS.

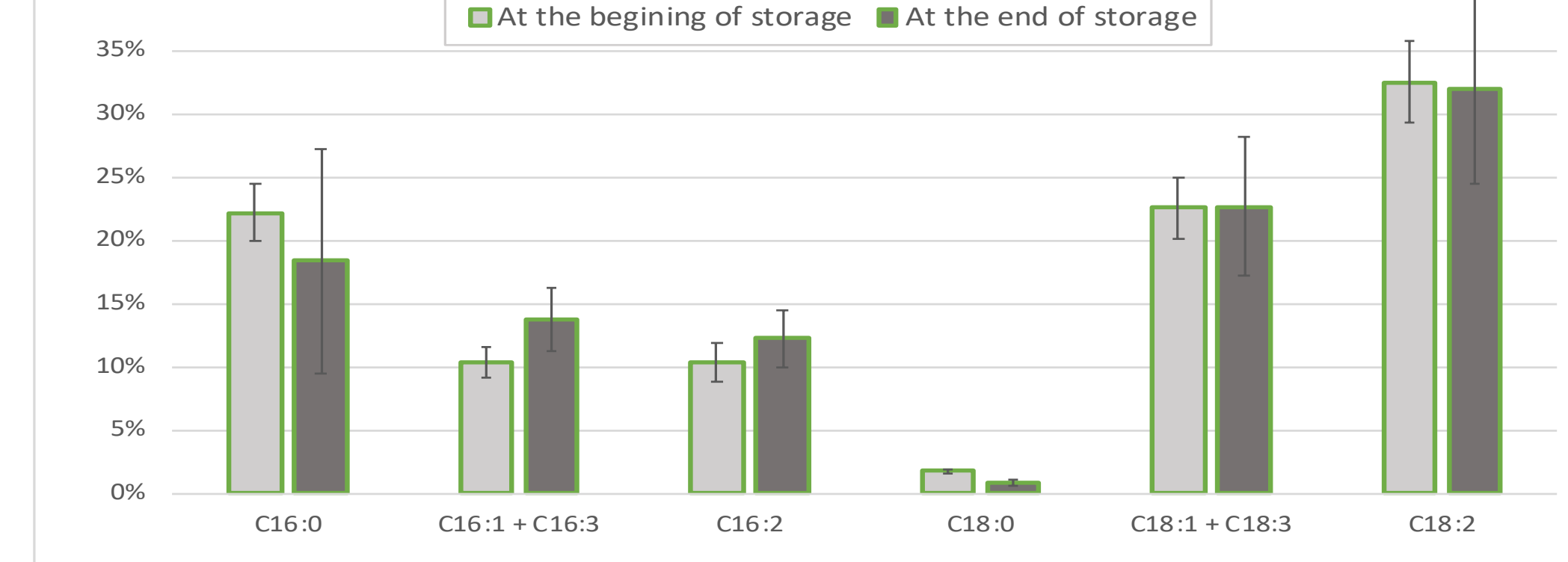
→ For cell densities above 45 gDW/L, the increase of FFA level was higher in proportion than the increase in cell concentration. This acceleration of the lipolysis phenomenon suggests an extension of lipolysis to other molecular forms of lipids that should be studied in more details.

Effect of DAS on the fatty acid profile

Fatty acid profiles of released fatty acids during dark anoxic storage in cells harvested during growth phase (% mol)



Fatty acid profiles of released fatty acids during dark anoxic storage in cells harvested during stationary phase (% mol)



The profile of the fatty acids released was stable between the beginning and the end of DAS

→ Whatever the time of harvest during cultivation, no significant evolution of the FFA profile could be observed during DAS.

CONCLUSIONS

Results showed that hydrolysis of microalgae lipids started when cells were harvested and concentrated, and that the level of free fatty acid released in the course of dark anoxic storage (DAS) could depend on the physiological state of the cells when harvested, *i.e.* in growth phase or stationary phase, and on the density of cells after concentration. In all cases the first stage of lipolysis that occurred during harvest resulted in an initial level of free fatty acids of about 12 mg of FFA per g of dry weight (DW) of biomass at the beginning of DAS. When cells were harvested during growth phase this level remained stable during DAS. When cells were harvested during stationary phase, a second important stage of lipolysis occurred at high rate, between 18 and 30h of DAS, resulting in a 3 times increase of FFA level, which then remained stable up to 120h of DAS. In addition, when cell density after concentration was superior to 45 gDW/L, it resulted in disproportionate increase in fatty acid release. **These data illustrate that lipolysis during wet storage is a complex phenomenon of which determinant factors are not fully elucidated. Here the results suggest that both the physiological state of microalgae cells and some parameters of the downstream processing are important to control the evolution of the molecular forms of the biomass lipids.** Further studies are ongoing to deepen our understanding of this challenging issue, to secure the quality of microalgae lipids for food applications.

REFERENCES : Balduyck, L., et al. (2016). Lipolysis in *T-isochrysis lutea* during wet storage at different temperatures. *Algal Res.* <https://doi.org/10.1016/j.algal.2016.07.003>; Balduyck, L., et al. (2017). Integrity of the microalgal cell plays a major role in the lipolytic stability during wet storage. *Algal Res.* <https://doi.org/10.1016/j.algal.2017.06.013>; Barouh, N., et al. (2024). Variations in *Chlorella* lipid content in commercial and in-lab produced biomass. *OCL.* <https://doi.org/10.1051/ocl/2024005>; Nami, F., et al. (2022). Real-Time NMR Recording of Fermentation and Lipid Metabolism Processes in Live Microalgae Cells. *Angew Chem.* <https://doi.org/10.1002/ange.202117521>