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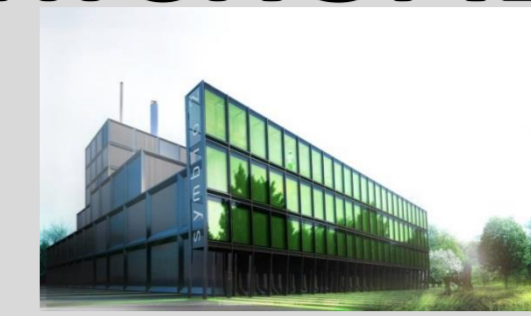
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SCREENING FOR ASTAXANTHIN PRODUCTION BY CULTURES OF MICROALGAE WITHIN INTEGRATED PHOTOBIOREACTORS FAÇADE

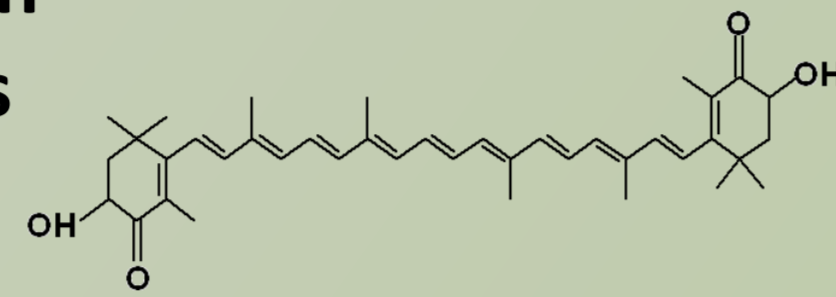


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Context

New closed photobioreactors designs for microalgae cultivation

- sustainably producing high yields of value-added products
astaxanthin 2000 \$ / kg (Lorenz & Cysewski, 2000)
- potent antioxidant
- prevention of age related macular degeneration (Yamashita, 2013)



- *Haematococcus pluvialis* astaxanthin up to 3 % DW (Lorenz & Cysewski, 2000)

Symbio2 project :

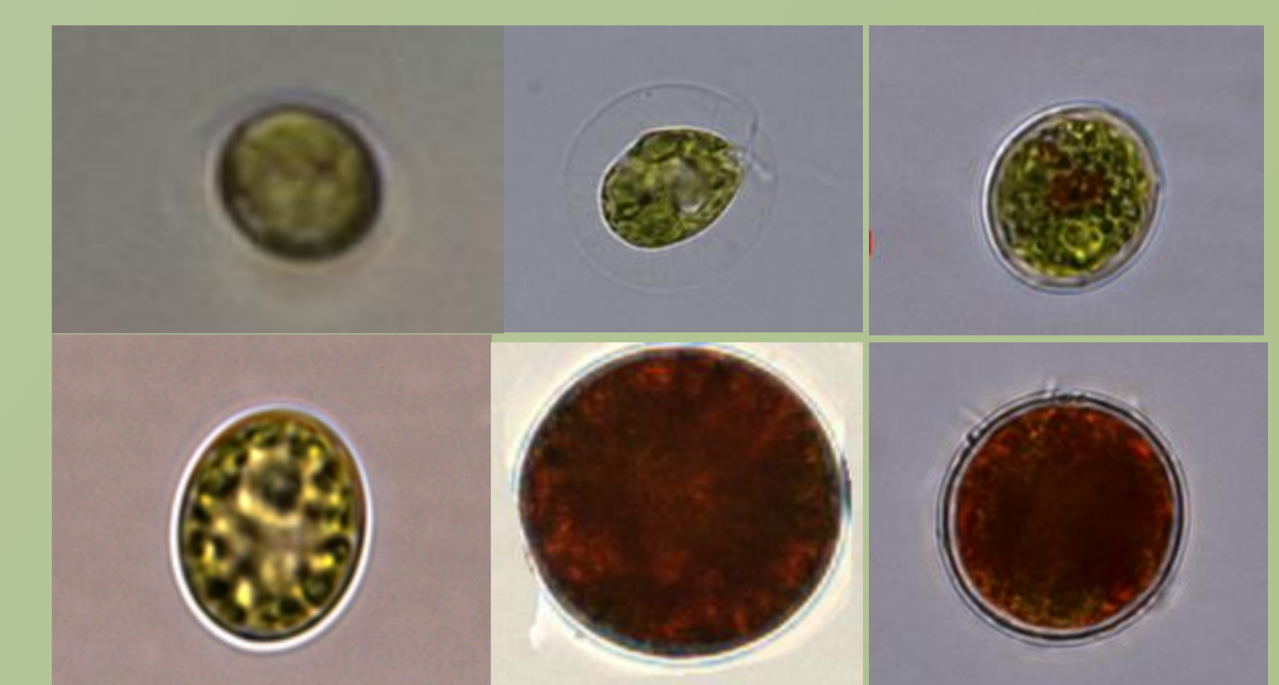
- Production of microalgae in biofacades photobioreactors (Pruvost *et al.*, 2015)
- Continuous-flow culture system
- No biofouling or biomass sedimentation ; control of encystement
- High astaxanthin productivities

Objectives

Use of microplates to :

- screen microalgae species for astaxanthin accumulation.
- compare the efficiency of different factors for triggering astaxanthin synthesis

Strain	Size (µm)	Cultivation in photobioreactors
M1	13-27	X
M2	5-8	X
M3	15-30	✓
M4	18-23	X



Whole cells spectrophotometric characterization

in situ measurements

Miniaturization of end-point assays

ex situ measurements

Limits of quantitation (LOQ) and saturation (LOS)

Signals (inter)calibration

Methodology

Cell incubation 96 and 24 wells microplates

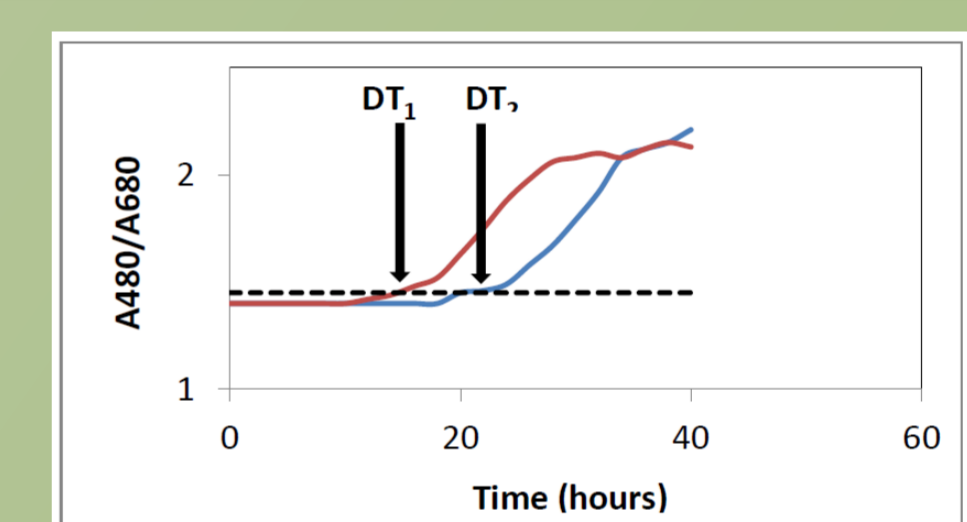
Control of environmental parameters ; PFD, T, rpm

Inoculum pre-treatments for batch incubations

Well-controlled initial nutrients status

Carotenoid induction

Identification of potent factors



Data treatment → Detection time (DT)
(Grizeau *et al.*)

Microalgae incubations

- Astaxanthin C₄₀H₅₂O₄
- C-sufficient medium with NaHCO₃

➢ Control of culture incubation :

Parameter	Value
Temperature (°C)	20
PFD (µmol photons m ⁻² s ⁻¹)	100
Light path (mm)	96 wells : 5 24 wells : 4,5

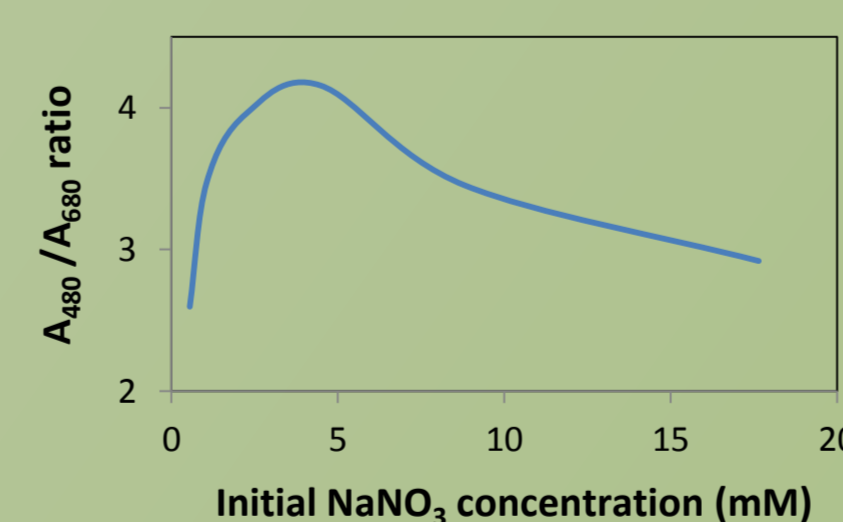
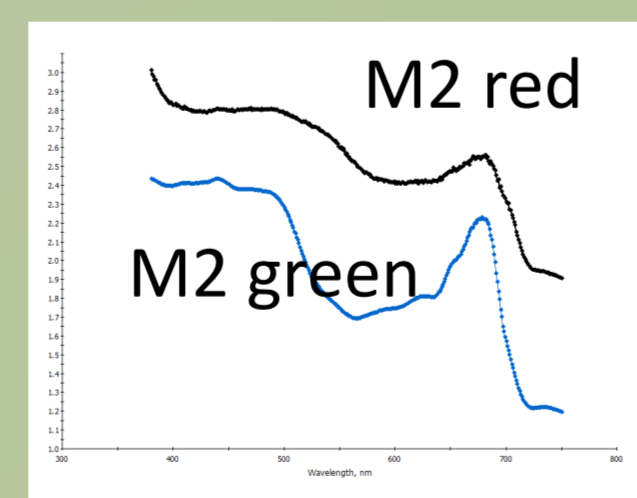
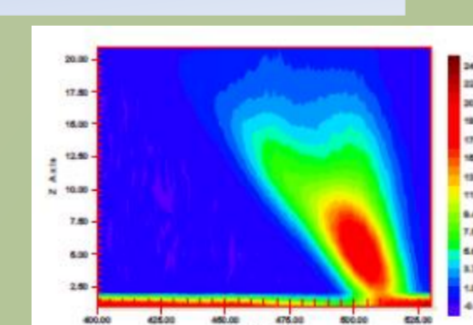
➢ incubation and reading



Multimode microplates reader

In situ measurements

- A₇₅₀ to estimate residual growth
- Fluorescence Ex₄₄₀/Em₆₇₀ = f ([chlorophylle])
- A₄₈₀/ A₆₈₀ ratio = f (time)
- Threshold of carotenogenesis = f (1/DT)

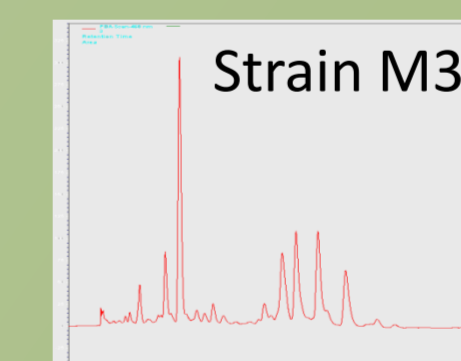


[NaNO₃]_{opt} for carotenogenesis in M2 cells

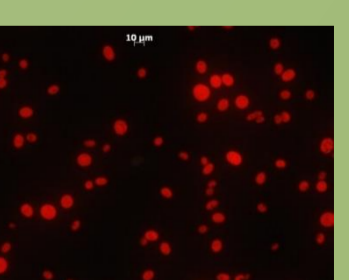
Ex situ measurements

Exemple 24 wells microplate :

- 200 µL extracted with DMSO :
spectrophotometry → [Total carotenoids]
HPLC analysis → [Astaxanthin]
→ carotenoid profile



- 2 µL photosynthetic activities :
→ Fv/Fm, NPQ and PI curves
- Microscopy / Image analysis
→ cell numeration
- Epifluorescence
→ cell viability
- Nitrate determination
→ conversion yields



Conclusions - perspectives

- Efficiency of a microplates format utilization for comparative studies of microalgae carotenogenesis
- Combining *in situ* and *ex situ* measurements → data acquisition for screening the efficiency of inducing factors on carotenogenesis
- Futur prospects ; improvements by using an automated workstation allowing semi-continuous cultures within microplates

