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



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Context

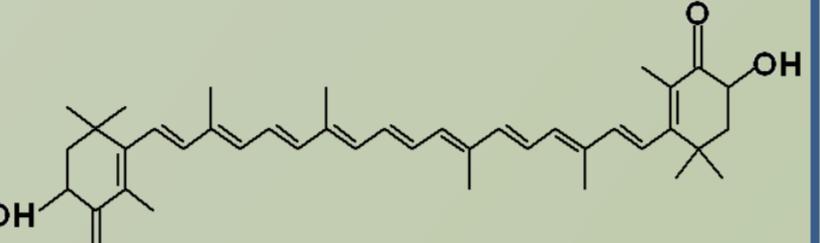
New closed photobioreactors designs for microalgae cultivation

→ 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

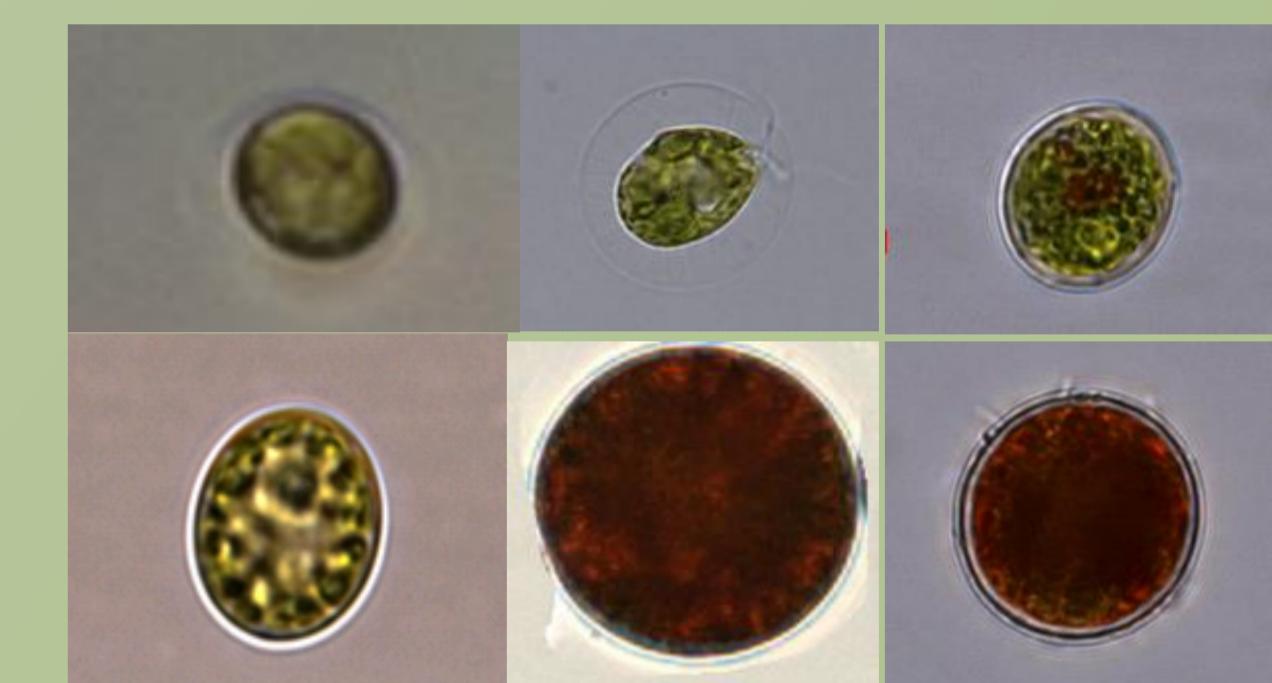
High astaxanthin productivities

Objectives

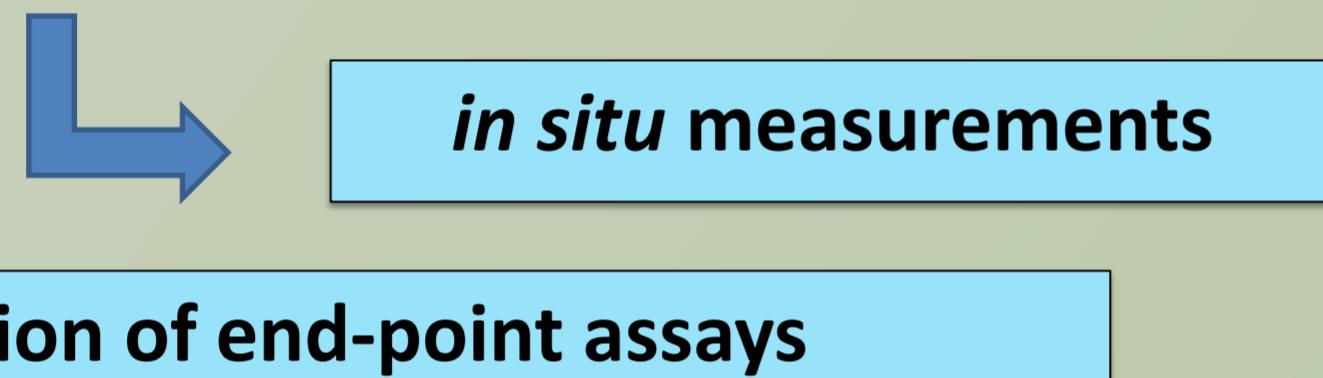
Use of microplates to :

- 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



Limits of quantitation (LOQ) and saturation (LOS)

Signals (inter)calibration

Methodology

Cell incubation 96 and 24 wells microplates

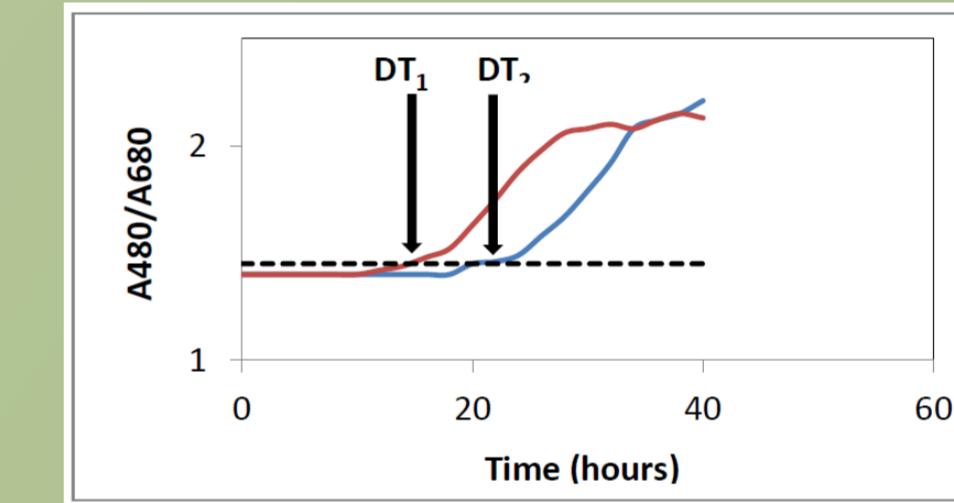
Control of environmental parameters ; PFD, T, rpm

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

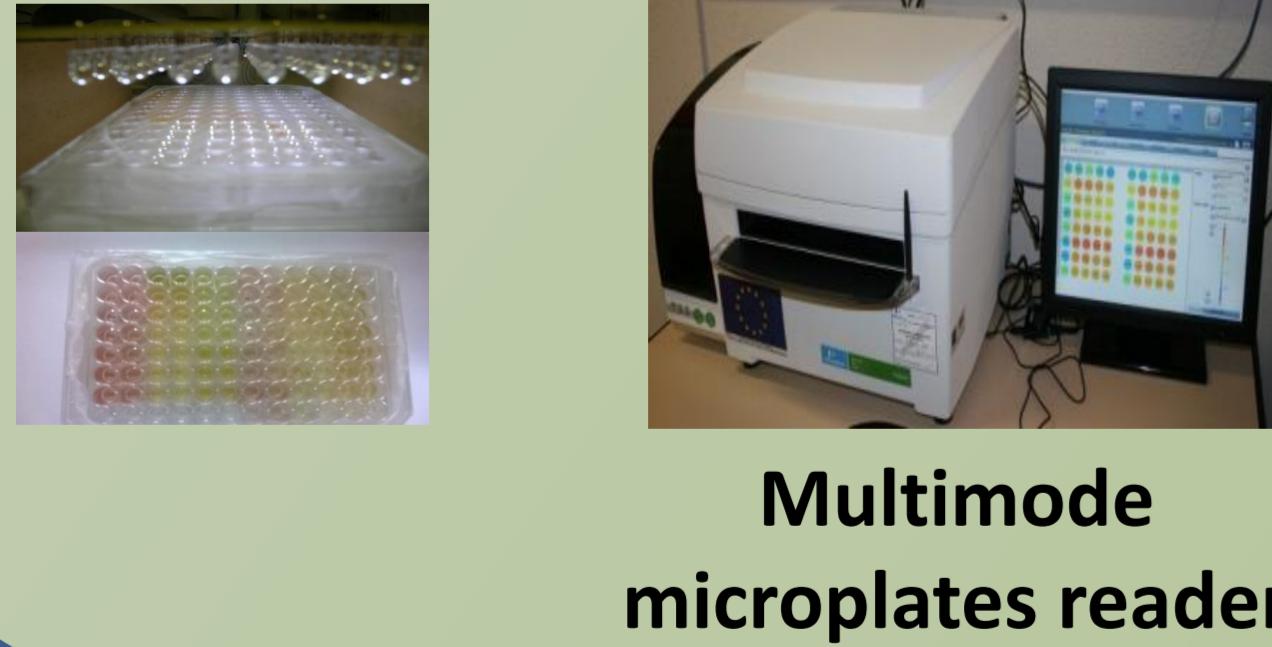
➤ Astaxanthin $C_{40}H_{52}O_4$

→ C-sufficient medium with $NaHCO_3$

➤ Control of culture incubation :

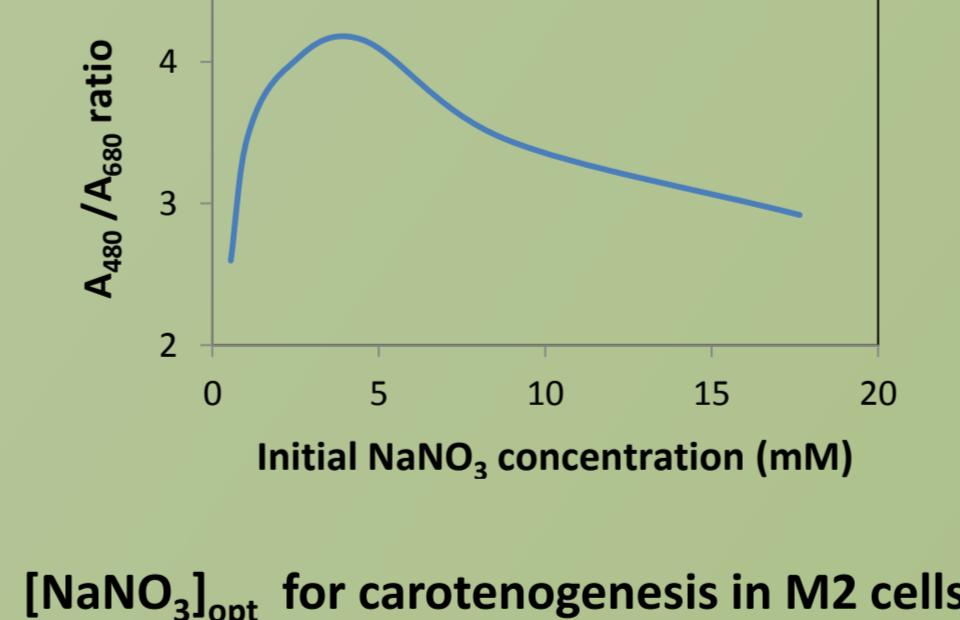
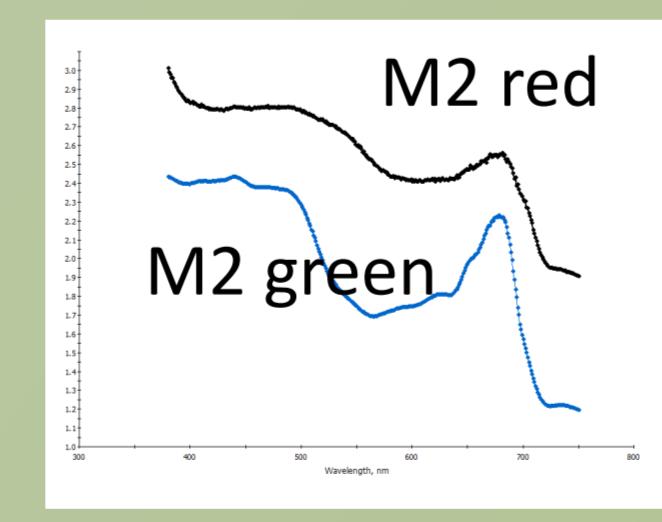
Parameter	Value
Temperature (°C)	20
PFD ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	100
Light path (mm)	96 wells : 5 24 wells : 4,5

➤ incubation and reading



In situ measurements

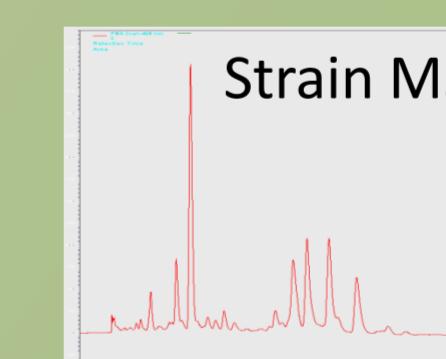
- A_{750} to estimate residual growth
- Fluorescence $Ex_{440}/Em_{670} = f$ ([chlorophyll])
- A_{480}/A_{680} ration = f (time)
- Threshold of carotenogenesis = $f(\frac{1}{DT})$



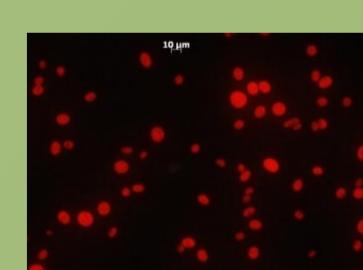
Ex situ measurements

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
- Future prospects ; improvements by using an automated workstation allowing semi-continuous cultures within microplates

