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MELISSA CONFERENCE 2020

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MICROALGAE R&D FACILITY

«EXPERIMENTAL FEEDBACK ON THE PILOT SCALE PRODUCTION OF *RHODOSPIRILLUM RUBRUM* AND CHALLENGES TOWARDS ITS INDUSTRIAL LARGE-SCALE PRODUCTION »

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CONTEXT

- ✓ Bacteria and microalgae are promising ingredients for space food.
- ✓ They grow exponentially and can provide many nutrients in an astronaut's diet
- ✓ They help to close the C and N cycle on a closed ecosystem like the ISS

Terrestrial Applications and Technology Transfer

What started as a food that astronauts could grow themselves is showing potential for lowering cholesterol levels around the world: space research has found a bacterium that can reduce cholesterol by half.

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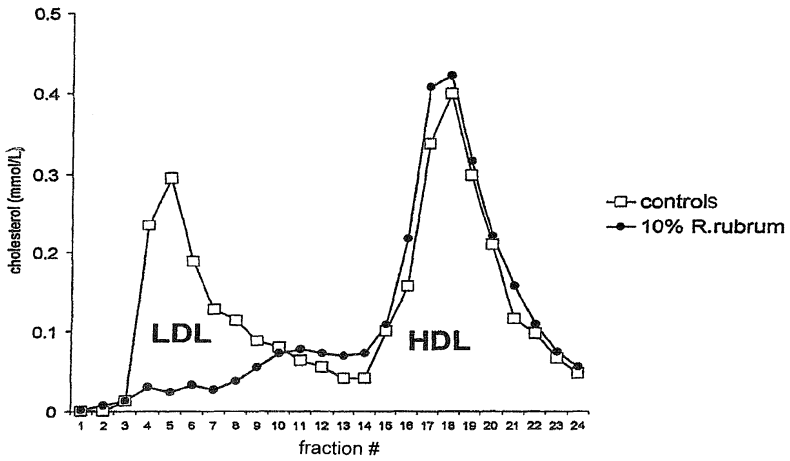
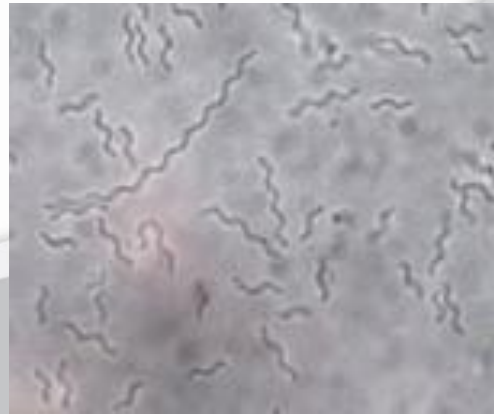


With ESA's support, spin-off company EzCOL BV was set up by IPStar BV, MELiSSA's technology transfer partner, to continue research and market the cholesterol-diminishing bacterium.



CHALLENGES

The aim of the study is to emphasize challenges towards the industrial production of an edible biomass from *Rhodospirullum rubrum* for human nutrition and cholesterol treatment.

Constraints:

- Heterotrophic growth condition for *R. rubrum* is driven under anoxic conditions, which means that no oxygen must be set on the culture medium.

The growth is then mainly driven by bacteriochlorophylls and carotenoids for light absorption.

- The complex metabolism of the strain induce a latency phase of up to 15 days at the start of cultures

- Culture medium reveals also a complex composition, and growth rate is found to be highly related to light intensity and pH value.

Therefore, setting an industrial production of *R.rubrum* requests to select the adequate culture technology, as well as a good understanding of *R.rubrum'*s metabolite response to culture conditions.

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MATERIAL & METHODS

- *R. rubrum* strain was provided from UniMons (2018)
- MELISSA culture media provided by Ezcol BV were studied and slightly modified to allow only one autoclavable mixture. Nutrients initial quantities were also optimized regarding the stoichiometry of the strain.
- Anaerobic condition were maintained by flushing Nitrogen
- Protocols for measuring pigments by visible spectroscopy and acetate by ion chromatography were developed. The stress index equal to the carotenoid/bacteriochlorophyll ratio. Bacteriochlorophyll represents growth and carotenoids represent stress.

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MAIN RESULTS – LAB SCALE

- Latency phase problem was elucidated thanks to self-seeding.
- Productivity is highly influence by light intensity and can be control with the stress index.
- *Rhodospirillum's* growth is reflected in the generation of IC, the consumption of acetate and a significant increase in pH.

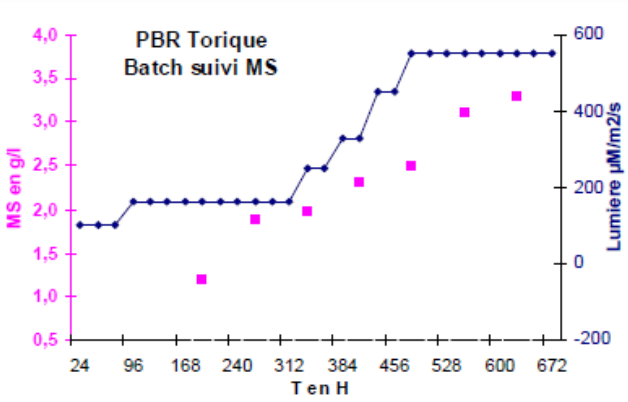


Figure A3.3 Evolution de la MS et de la lumière en cours de culture

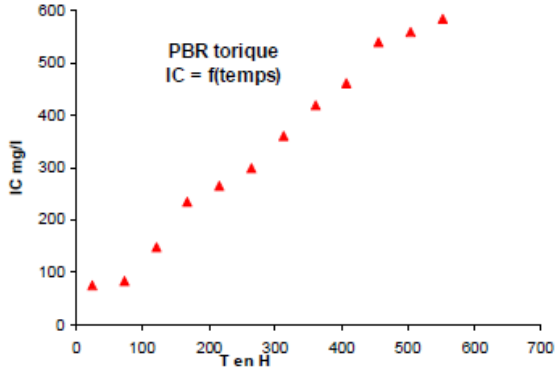


Figure A3.2a : Evolution du carbone inorganique en cours de culture

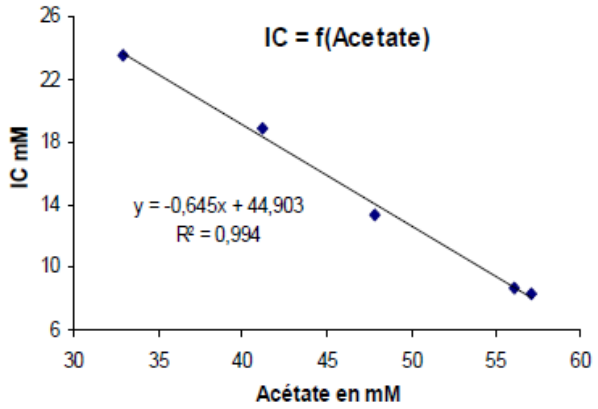


Figure A3.2b : évolution de l'IC en fonction de l'acétate

MAIN RESULTS – LAB SCALE

- Dry matter is related to the amount of Bacteriochlorophyll a. This allow a more precise follow of the productivity.
- At 560µmol.m^{-2}.s^{-1} maximum concentration was reach at 3.3 g/L DW (photolimitation)
- Productivity reach 0.9 g/L/day at 560µmol.m^{-2}.s^{-1} thanks to self seeding and vigorous mixing
- The carotenoid content is shown proportional to the content of bacteriochlorophyll a

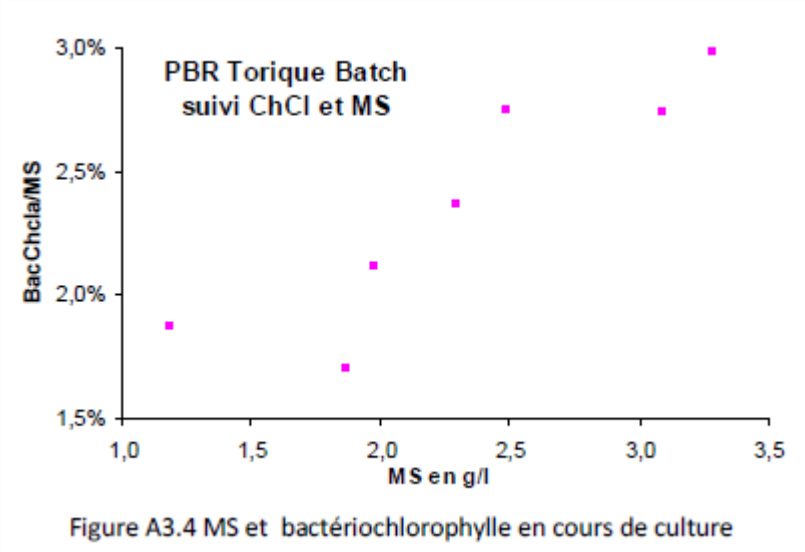


Figure A3.4 MS et bactériochlorophylle en cours de culture

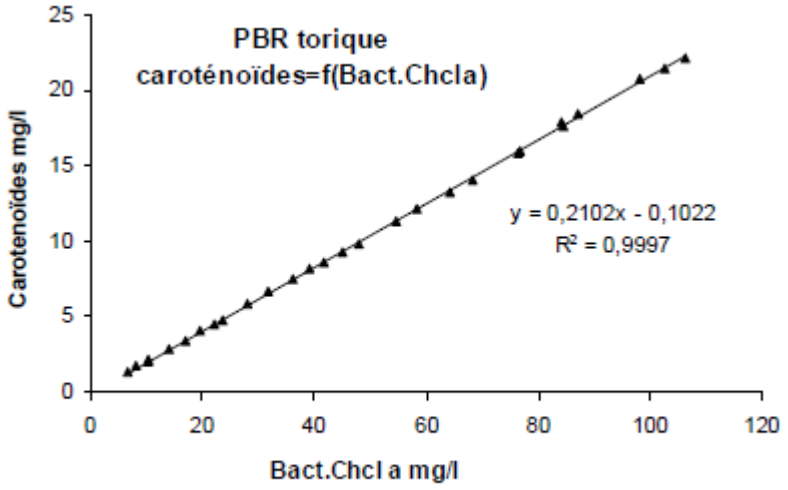
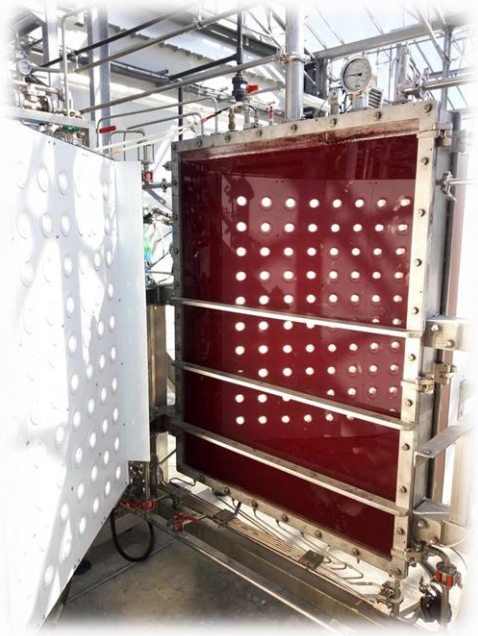


Figure A3.5 Caroténoïdes et bactériochlorophylle a au cours du temps

MAIN RESULTS – PILOT SCALE

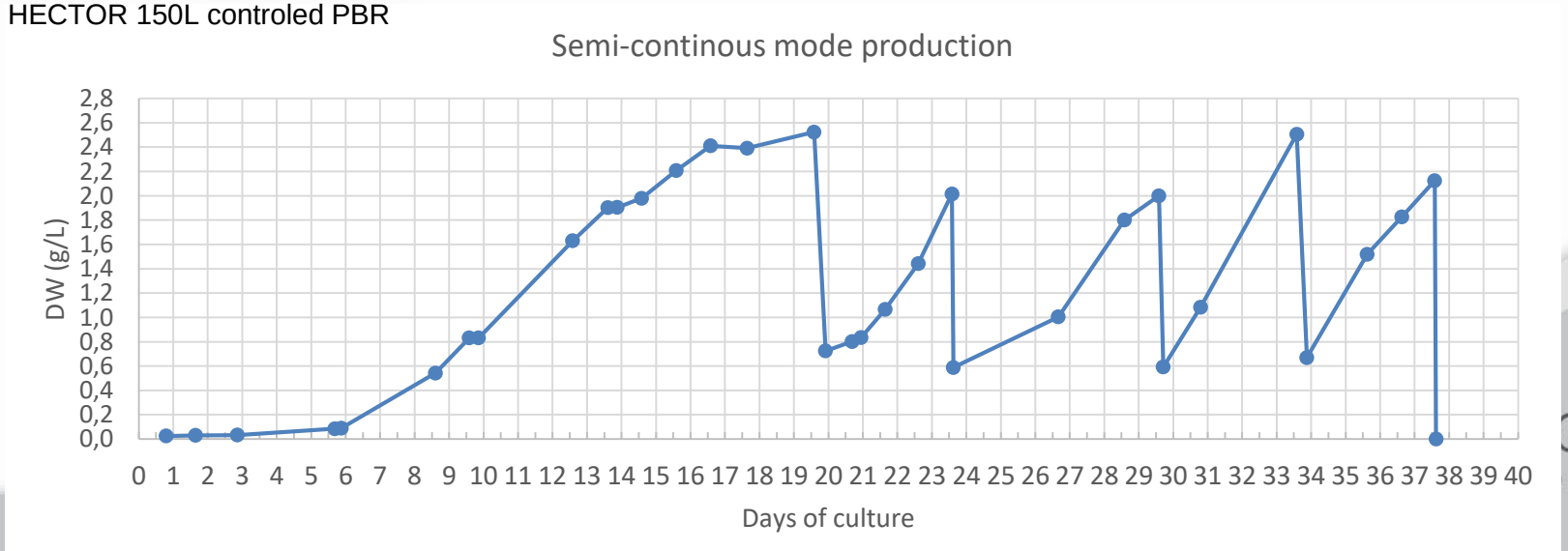


HECTOR 150L controled PBR

- Inoculation with 6L of concentrate culture from the Torus PBR.
- Latency phase : 7 days
- C_{max} = 2,56 g/L
- Light intensity were progressively increased up to 900 µmol.m^{-2}.s^{-1}
- Productivity in exponential phase : between 0,25 g/L/day and 0,50g/L/day (depending on batch)

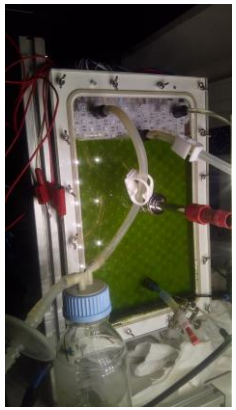
- 1.4 kg DW were harvested from this semi-continuous mode production.

→ **Identified bottleneck : Biofilm / biofouling**



INTENSIFIED PBR : DESCRIPTION OF ISOS PBR

ISOS PBR



Isos is a lab scale PBR design to study the maximal potential of microalgae growth, developed by the GEPEA laboratory.

The dark fraction is really low (thin layer) and under control condition, we demonstrate a higher productivity than standard PBR (avoid photolimitation).

The thin layer allows a better hydrodynamic, thus the bubbling avoid creation of biofilm and allow a strong agitation.

Experimental results on model strain:

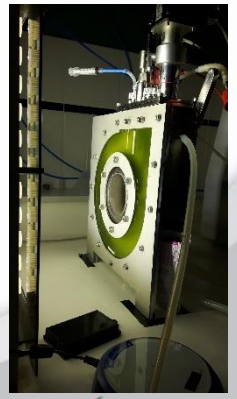
In the same operating conditions (light 200µmol/m²/s, dilution rate 0,033h-1, temperature, pH), we experimentally demonstrate a higher productivity by 8 times in our intensified PBR (ISOS) than standard PBR (torus).

	Concentration (g/L)	Surface productivity (g/m²/j)	Volume productivity (g/L/j)
Torus PBR	0,40	14,3	**0,32**
ISOS PBR Experimental	3,22	7,4	**2,56**
ISOS PBR Theoretical	*6,22*	*14,3*	***4,95***

X 8

X 16

TORUS PBR



Results from J. Tallec study

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TECHNOLOGY EVALUATION FOR *R. RUBRUM*

- Melissa medium modified
- Nitrogen Bullage
- pH 8 regulation
- 30°C
- Continuous flow: PFD= 100µmol/m2/s; D=0.015h-1

Second test was successful thanks to the management of the temperature and the use of an active inoculum for the seeding.

	Concentration (g/L)	Surface productivity (g/m²/j)	Volume productivity (g/L/j)
Torus PBR 220µmol/m²/s	2,15	12,5	**0,26**
ISOS PBR Experimental 100µmol/m²/s	2,38	2,47	**0,86**
ISOS PBR Theoretical 220µmol/m²/s	*6,22*	*12,5*	***4,35***

No parameters optimisation were done on this preliminary study.

Results from R. Touchard studies

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CONCLUSION AND PERSPECTIVES

Those studies have shown a strong different from this bacterium behavior compared to known microalgae or cyanobacteria.

Understanding of how *R. rubrum* metabolism is reacting to external parameters allows to improve productivity and reproductibily.

Possible scaling up of the culture has been demonstrate on HECTOR PBR, but still can be optimized in terms of latency phase, mixing and management of biofilm.

The use of an intensified culture technology (ISOS) has been demonstrated on this strain, and could allow a viable industrial production.

More study needs to be done on the parameters optimization for the ISOS technology to reach a solid industrial production protocol.

CAPACITÉS
Infiniment possible

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Thank you for your attention