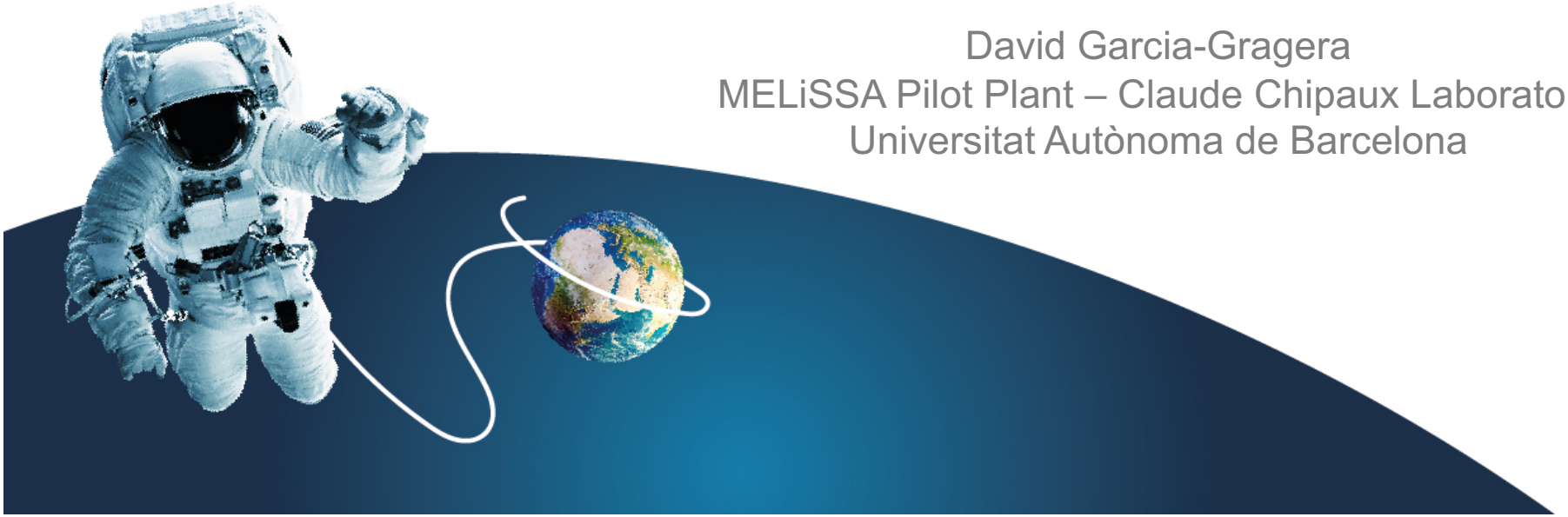
MELiSSA
MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

Dynamics of *Limnospira indica* continuous culture in and air-lift photobioreactor



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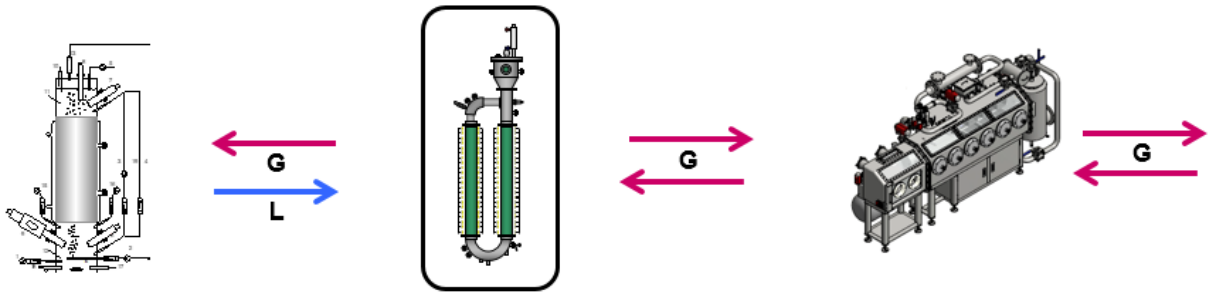
1. Background and objectives

Background and context

MELiSSA is a developing Technology for generative life support system to enable long-term human space missions.

MELiSSA Pilot Plant mission:

- ✓ Demonstration of MELiSSA concept
- ✓ Stepwise Integration of each element in the loop
- ✓ Capitalising the knowledge



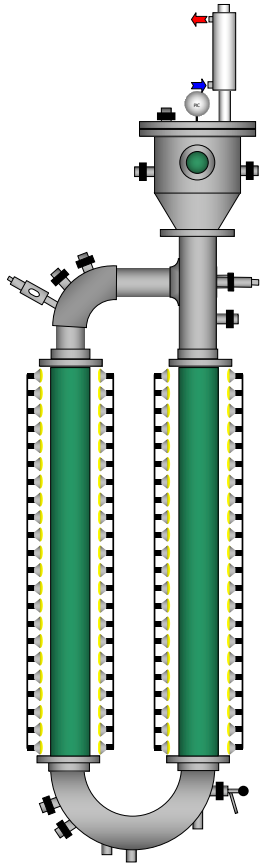
C4a Photobioreactor

- ✓ **Technology**: 83L external-loop air lift Photobioreactor
- ✓ **Biological component**: *Limnospira indica* axenically cultivated

Functions

- **Air revitalization**
- Edible material generation (50-70% protein content)
- Respond rapidly to dynamic changes of MELiSSA loop

Technology development & Optimisation



Lighting Technology upgrade

Halogen lamps

LED based

- ✓ Lighting system upgraded
- ✓ LED based Technology
- ✓ Higher Photon Flux Density (up to 1700 $\mu mol \cdot m^{-2} \cdot s^{-1}$)
- ✓ Better quality spectrum

Culture performance characterisation

Research Objectives

Work related with C4a is focused in the following points:

1. **Characterise** the performance of ***Limnospira indica*** with the LED-based illumination system
2. Investigate the **dynamics** of C4a culture under different illumination conditions during **long-term continuous operation**
3. Understand the **molecular basis** behind of the process performance
4. Definition of the best operational conditions for **O_2 production** in an **integration** strategy context.

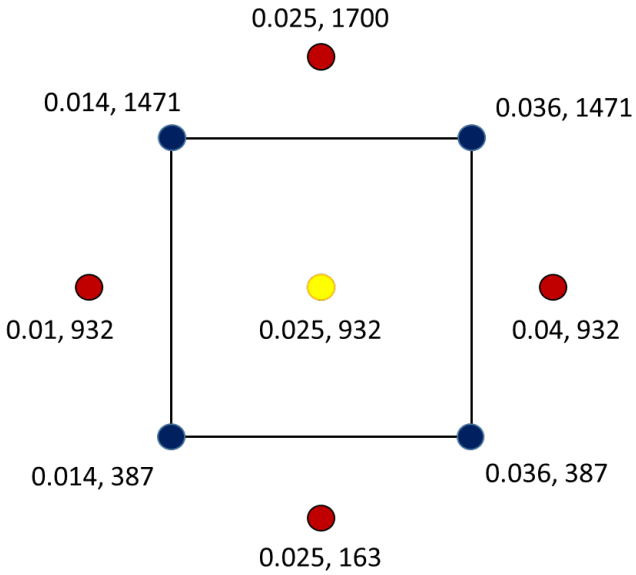
2. Experimental design

Experimental design

Make use of experimental **Design of Experiments methodologies (DoE)** to explore the relationship between independent and response variables → understand how the system behaves to work under optimal conditions

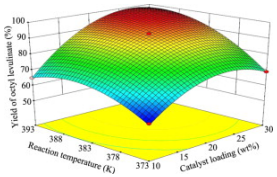
Variables for optimization
- Biomass [X]
- O_2 production

Manipulated variables
- *D* (h^{-1})
- PFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)



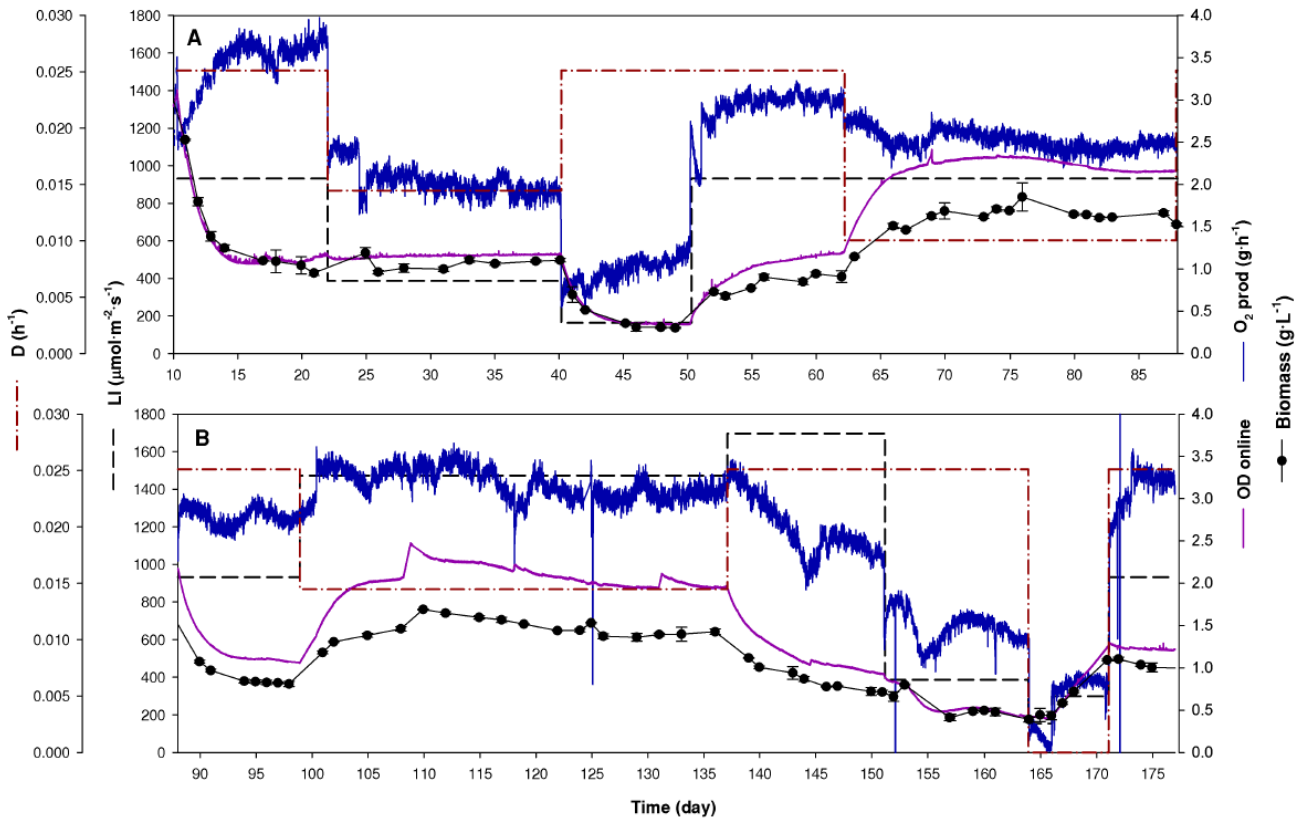
CCD

	Min	Max
D	0.010	0.040
PFD	160	1700



3. Results

Process Performance



Long-term operation: 177 days

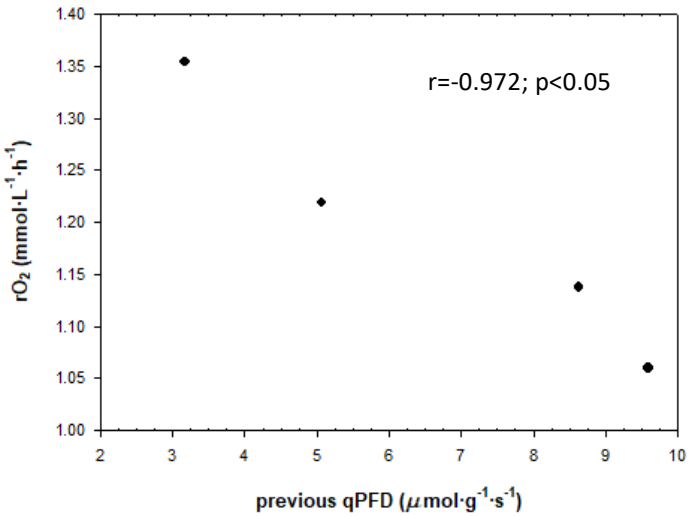
Steady state (6 HRT)

System Robustness

Process Performance

Steady-state values

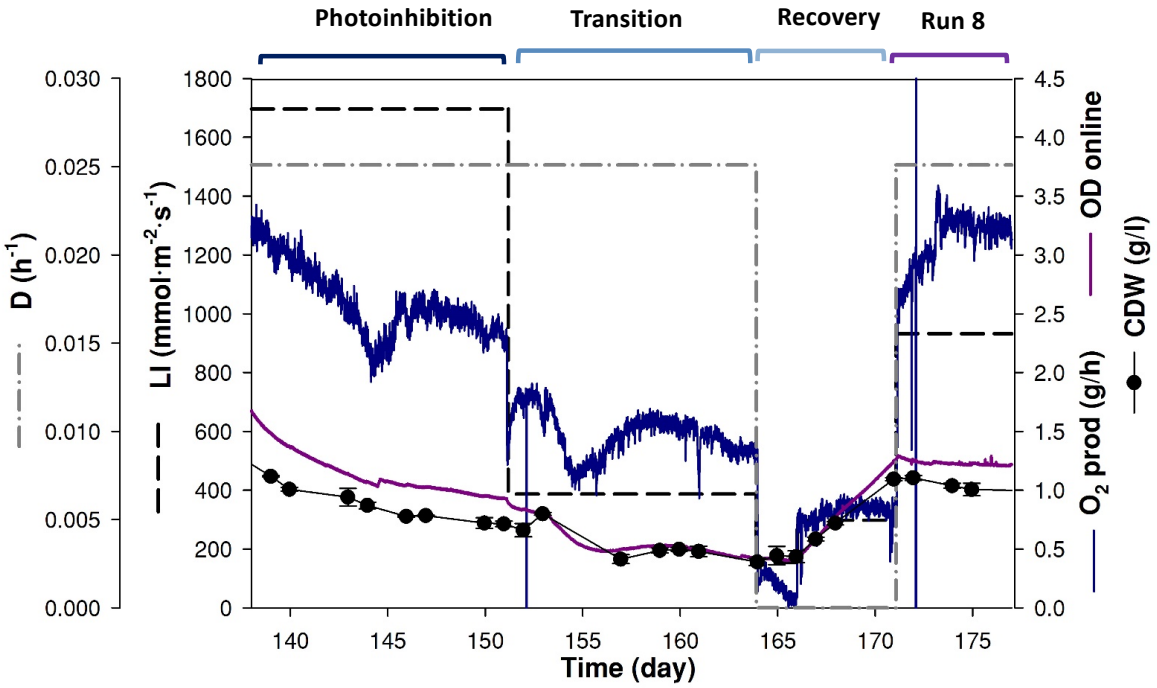
Run	PFD (µmol/m2/s)	D (h^{-1})	X (g/L)	*qPFD* (µmol/g/s)	rO_2 (mmol/L/h)	qO_2 (mmol/g/h)
1	932	0.025	1.05±0.07	15.14	1.35±0.04	1.29±0.12
2	387	0.014	1.07±0.04	6.12	0.73±0.04	0.62±0.06
3	163	0.025	0.32±0.02	8.63	0.40±0.02	1.13±0.12
4	932	0.025	0.90±0.04	17.56	1.14±0.03	0.99±0.08
5	932	0.01	1.65±0.10	9.59	0.93±0.03	0.41±0.06
6	932	0.025	0.82±0.01	19.22	1.06±0.03	0.95±0.05
7	1472	0.014	1.42±0.05	17.61	1.14±0.03	0.81±0.06
8	932	0.025	1.01±0.02	15.64	1.22±0.03	1.20±0.05



Steady-states multiplicity

Process performance

Photoinhibition



✓ **Photoinhibition**
- No stability after 6 HRT
- Continuous drop O2 and Biomass
- Kinetic regime (γ > 1)
- Yellowish appearance

✓ **Transition**:
- Recovery of the cells is not achieved
- Changes in O2 production and biomass
- Cells are still under stressful conditions
- Yellowish appearence

✓ **Recovery**
- Switch to batch mode
- Dim light → 150/300 µmol/m^2/s
- **Reversibility** is confirmed by central condition

Process Performance

Photoinhibition

- ✓Excess of P generated due to recycling of *e* from PSI to plastoquinone
- ✓Overexcitation of PS → degradation of Phycobilisome proteins
- ✓P/2e$^-$ >1.5 → Kinetic regime
- ✓Potential photoinhibition in kinetic regime (not bearable by metabolism)

Cornet JF, (2007)

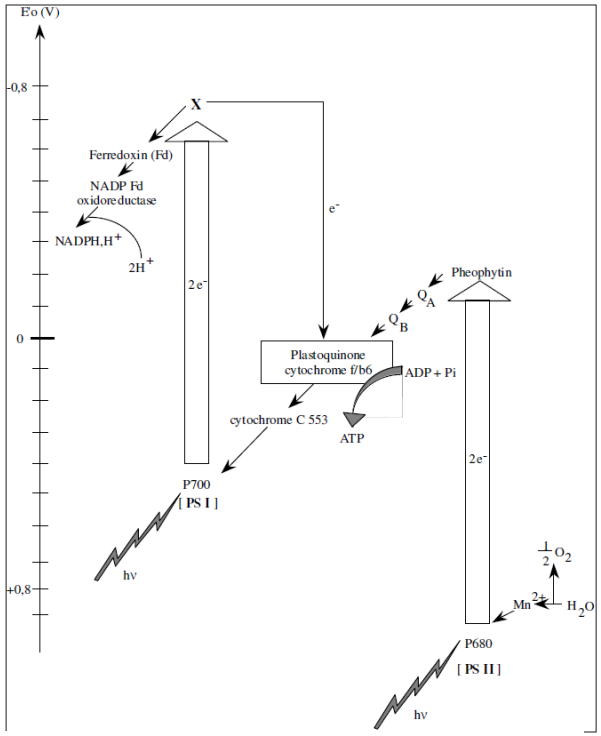


Figure IV.1 : Schéma en Z de la photosynthèse chez les cyanobactéries (d'après Cornet *et al.*, 1998).

Cornet JF, (2007)

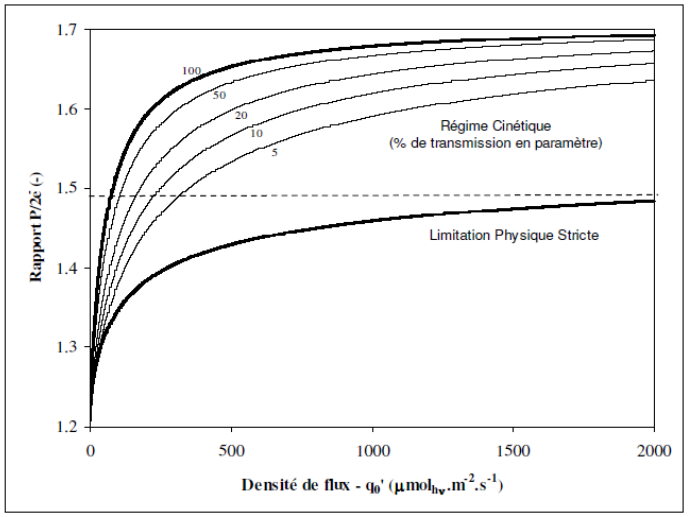
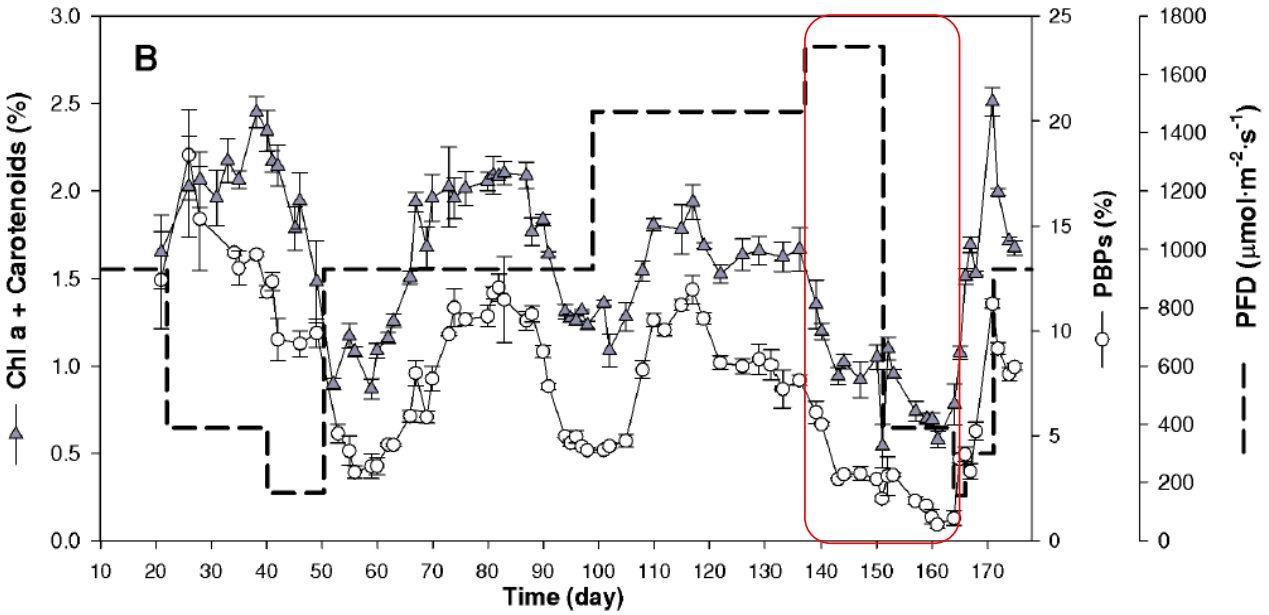


Figure IV.3 : Tracé du rapport P/2e$^-$ en fonction de la densité de flux incidente q_0' (éqs IV.3.18, 40-42) dans le cas d'un réacteur rectangulaire éclairé d'un côté avec un champ quasi-collimaté. Les deux régimes de fonctionnement du PBR sont illustrés par un faisceau de courbes comprises entre les états limites en trait épais. La valeur maximale du rapport P/2e$^-$ pouvant être atteinte en limitation physique par le transfert de rayonnement est indiquée. Pour illustrer le fonctionnement en régime cinétique, différentes situations ont été choisies en faisant varier la transmission (indiquée en paramètre) entre 5 et 100%.

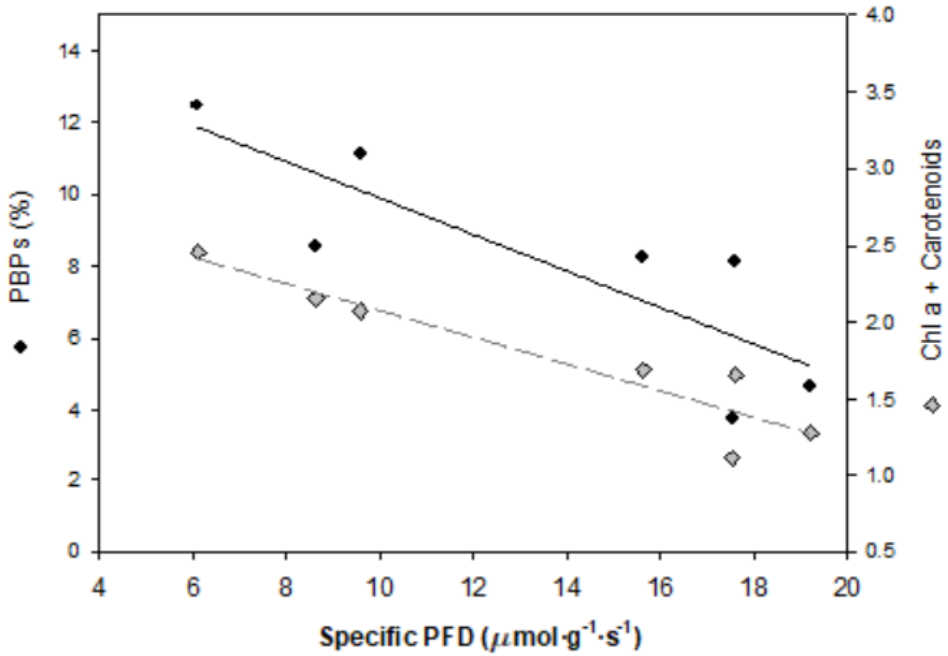
Molecular composition - Pigments

- ✓Pigments changes depend on process conditions
- ✓PBPs and Chla follow the same behaviour
- ✓↓ pigment during photoinhibition: 140-164 days
- ✓Culture colour in agreement with pigment content

Cells are exposed to ↑ *PFD*



Molecular composition - Pigments



Influence of qPFD

✓Statistical correlation:

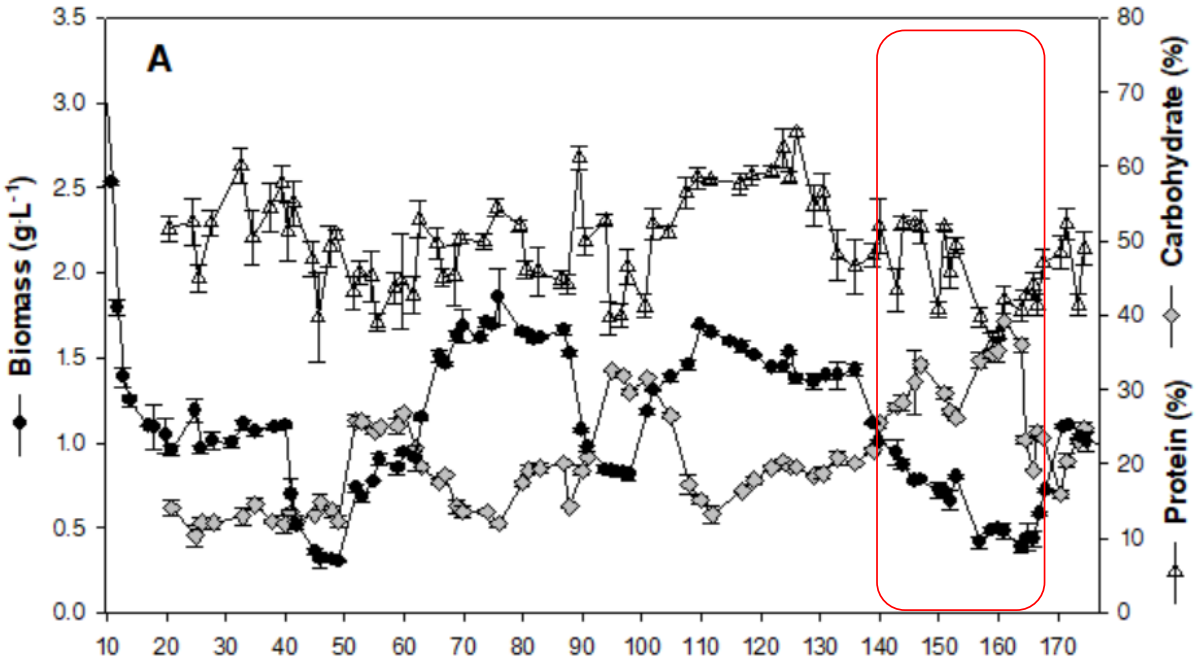
- PBPs: r=-0.88; p<0.05
- Chl a: r=-0.936; p<0.05

✓Regultation of mechanisms related to light absorption

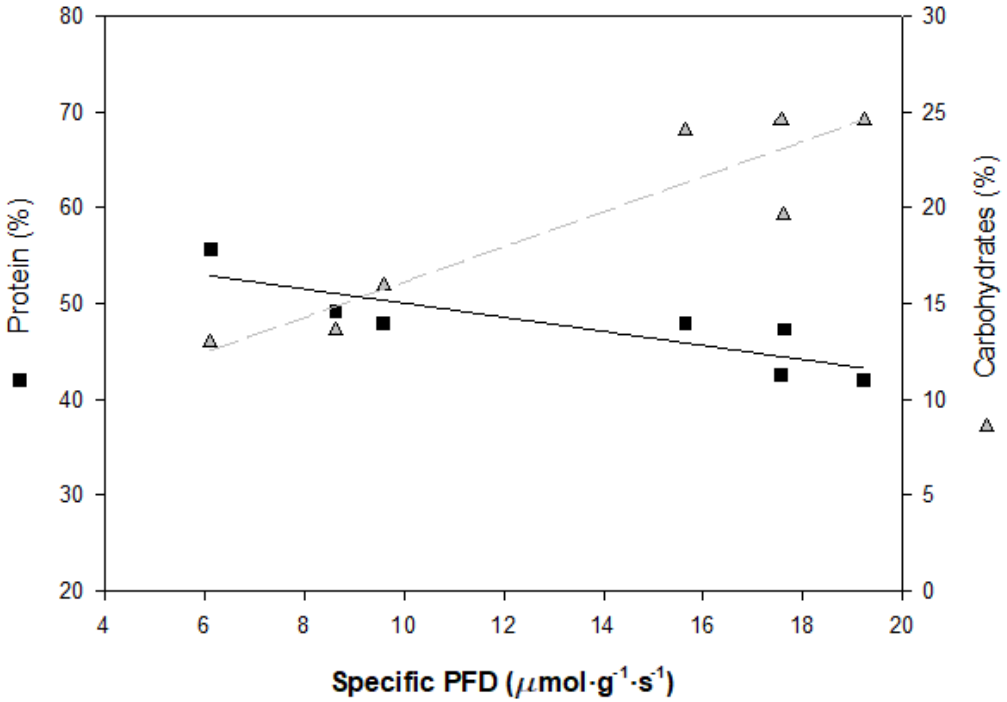
Cell composition directly depends on light availability rather than absolute light

Molecular Composition – Protein & CH

- ✓Protein variability is less significant
- ✓Protein ranges 40-60%
- ✓Carbohydrate variability is remarkable
- ✓CH content: 10% up to 40%



Molecular composition – Protein & CH



Influence of *qPFD*

✓Statistical correlation for protein and CH:

- **Protein**: r=-0.82; p<0.05
- **CH**: r = 0.925; p<0.05

CH accumulation normally related with stress conditions

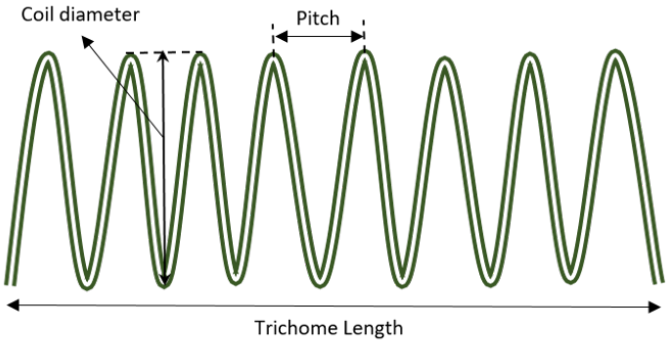
- Nutrrient
- P
- Excess of light

High P/2e$^-$ → EPS formation (fraction of CH)

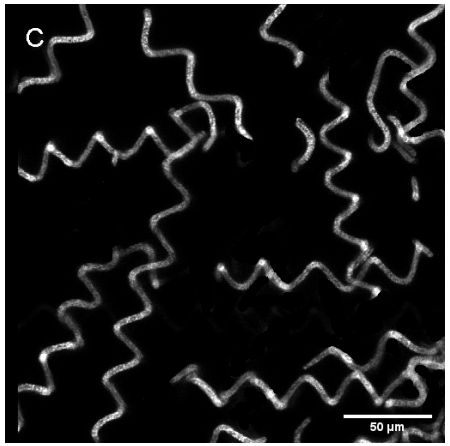
Cell Morphology changes

Limnospira cell morphology is a mirror of the physiological status of the cell:

- **Trichome length**: related to vitality
    - Short trichome: cells under stress
    - Long trichome: high vitality
- **Helix pitch**: decreased when exposed to UV-A/UV-B (self-protection mechanisms)

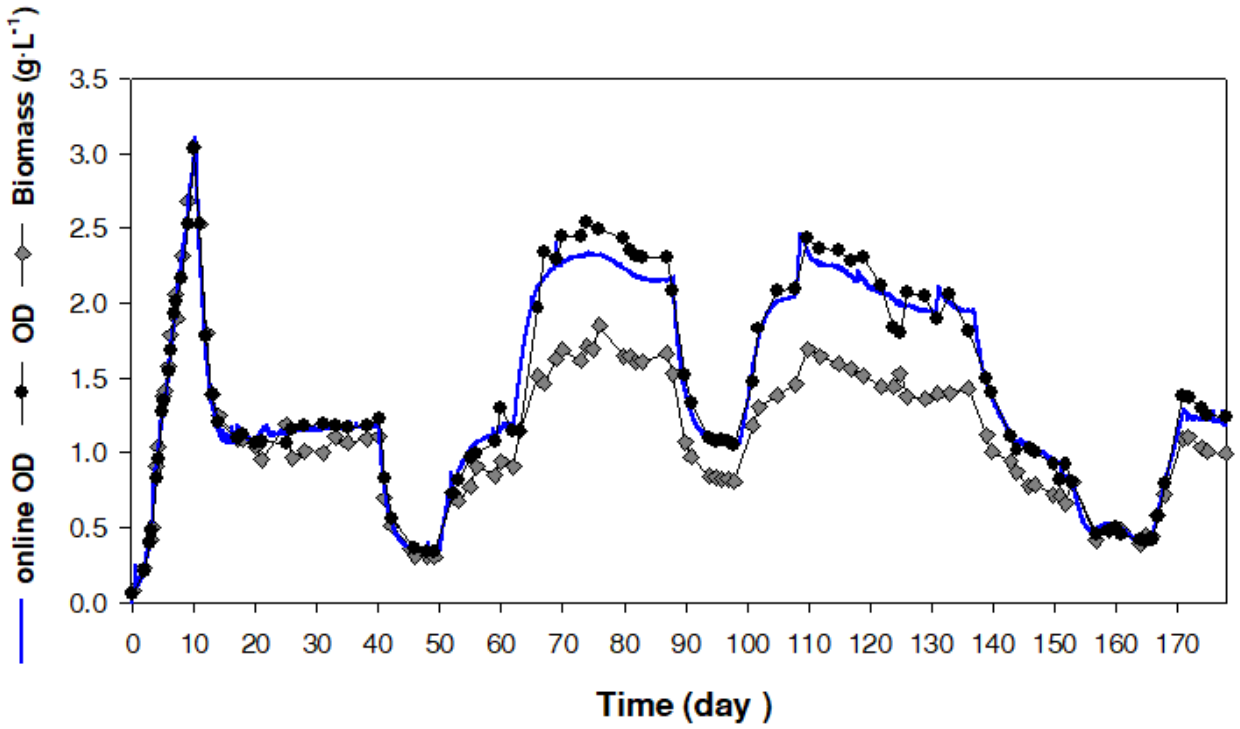


Microscope observation



Cell Morphology changes

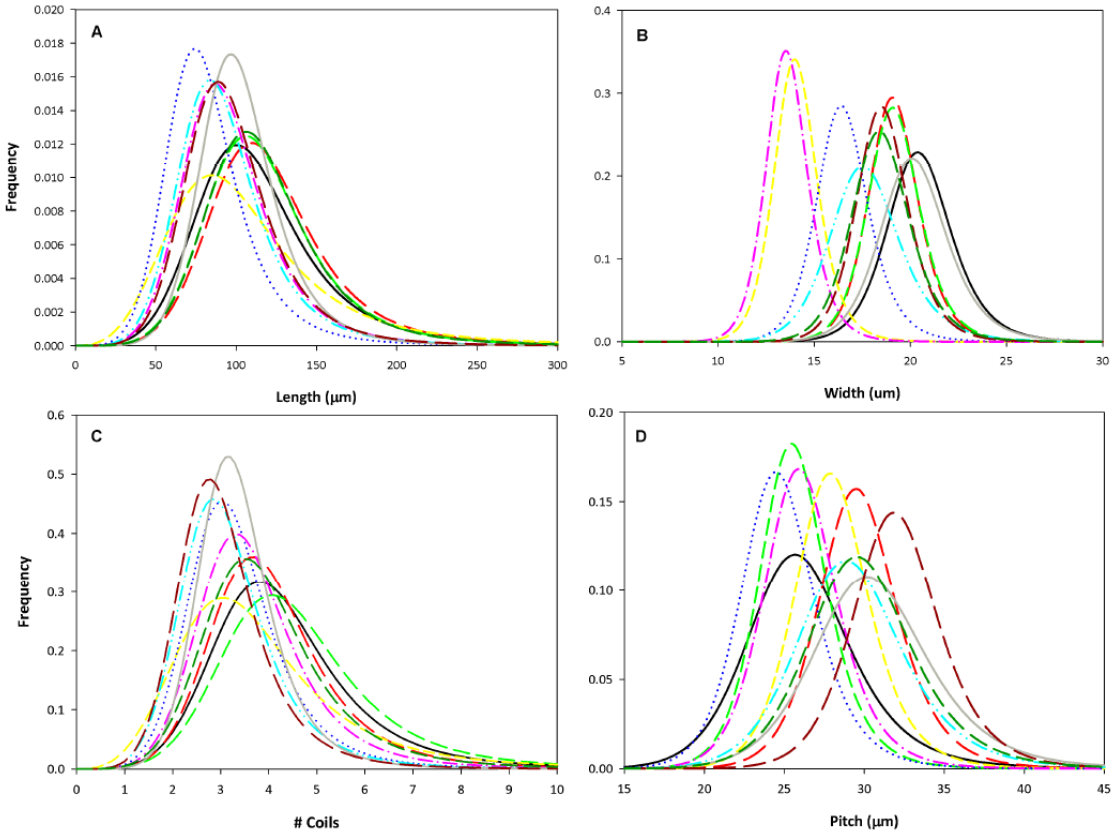
The first consequence of morphology changes is detected at on-line monitoring level



- CDW/OD ratio is not maintained constant
- Perfect fitting off-line and on-line values
- CDW/OD ratio: 1 – 0.67
- Direct cause of CDW/OD ratio variation is not identified

Cell Morphology changes

What morphological changes are responsible of the observed phenomena?

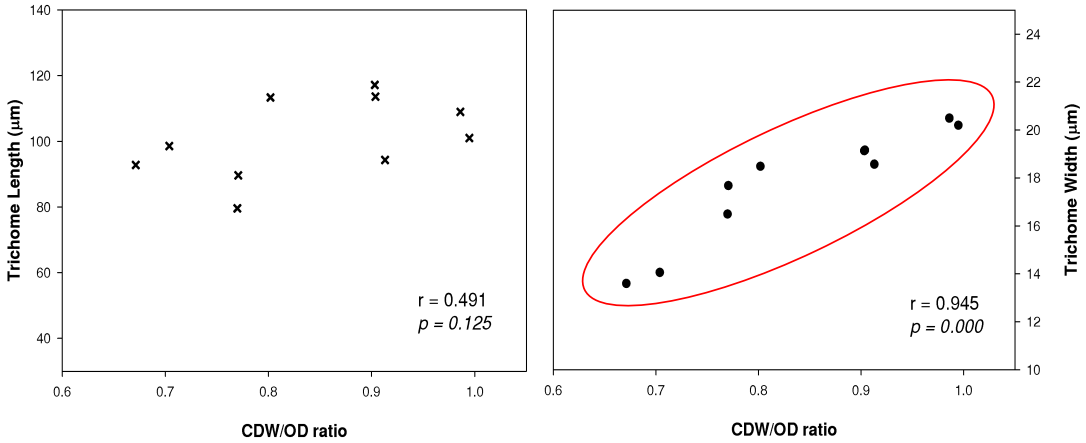


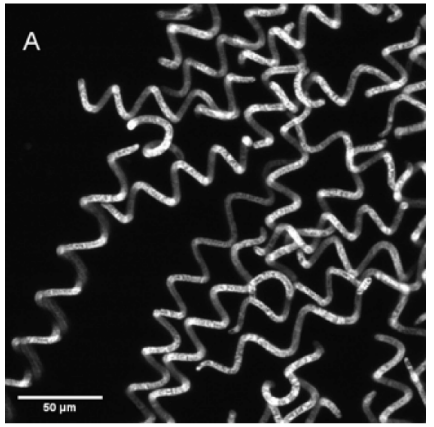
Distribution analysis for different size parameters

$$S = \frac{x^a}{k^a + x^a} = \frac{dS}{dx}$$

Parameter	*K* range	Typical values
Length	117-80 µm	100 – 3000 µm
Width	20 – 13 µm	20 - 100 µm
# coils	4.4 – 2.9	2 – 20
Pitch	32 – 24 µm	10 - 150 µm

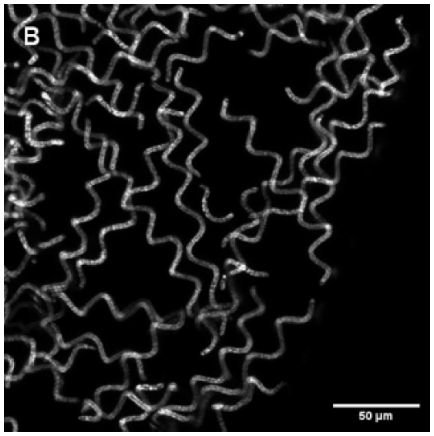
Cell Morphology changes





k = 20 µm

k = 13 µm



Cell Morphology changes

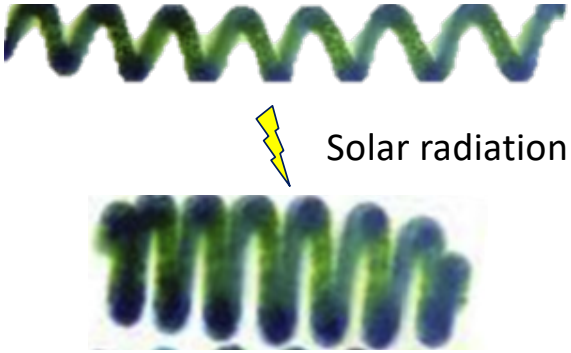
What are the causes?

Morphological changes in *Limnospira* in **nature** can be related to:

- Environmental stressful conditions
- Limitation of nutrients
- Excess of light radiation (solar)
- Salinity, pH, Temp.

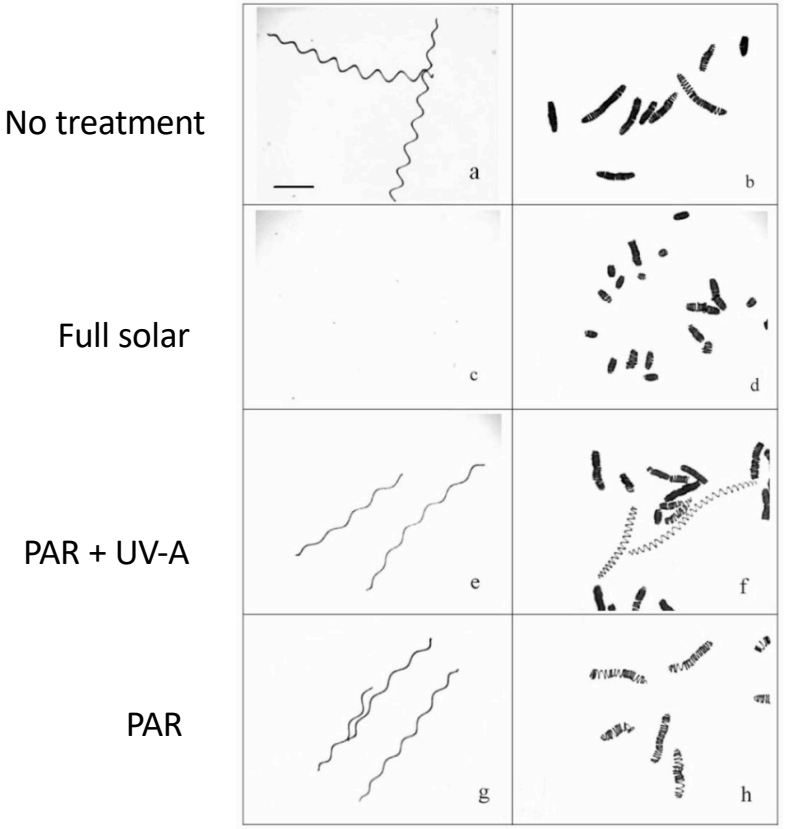
➢Trichome length

➢Helix Pitch



Solar radiation

Hongyan W. (2005)



Cell Morphology changes

What are the causes?

Variable	Correlation	Length		Width		Pitch		Coil Counts	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
qPFD	Person's	-0.503	0.138	-0.124	0.733	-0.407	0.244	-0.101	0.782
	Spearman's	-0.697	0.025	-0.382	0.276	-0.345	0.328	-0.358	0.31
PFD	Person's	-0.492	0.148	-0.538	0.109	-0.315	0.375	-0.273	0.446
	Spearman's	-0.54	0.108	-0.54	0.108	-0.263	0.462	-0.266	0.53

Only *Length* presents a non-linear correlation with *qPFD*

- Minimum length is 67% of the maximum
- No drastic changes
- Helix pitch is not affected

1. Current experiments avoid the use of UV radiation (only PAR → 400-700 nm)
2. Cells photoinhibited when exposed to high *qPFD,* but no morphology changes
3. Spiral breackage is not observed → accumulation of ROS is considered limited

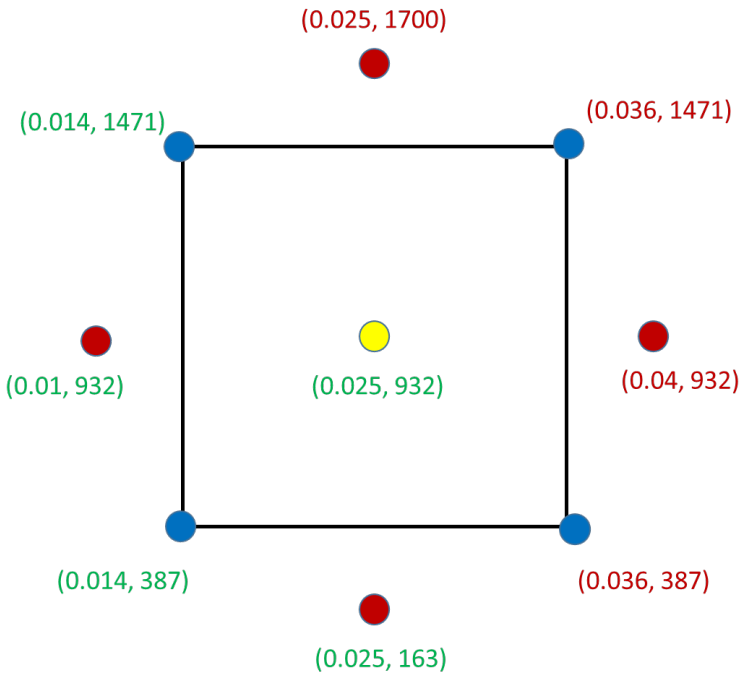
CONFIRMS REVERSIBLE PHOTOINHIBITION

4. Conclusions

Conclusions and future work

1. Cell culture response to changes in *D* and *PFD* have been investigated from different angles (rO_2, composition, morphology)
2. ***qPFD* (specific Photon Flux Density)** identified as the key parameter governing light availability and performance
3. Continuous operation is very **stable** and **robust** in the range D = 0.01-0.025 h^{-1}; PFD = 163 – 1472 $\mu mol \cdot m^{-2} \cdot s^{-1}$.
4. **Photoinhibition** observed under kinetic regime (X<1 $g \cdot L^{-1}$ and PFD=1700 $\mu mol \cdot m^{-2} \cdot s^{-1}$)
5. **Photoinhibition is reversible** under dim light → robustness of the system confirmed by limited changes at morphological level
6. **Molecular composition** is governed by *qPFD*.
7. Further studies to optimise the response of the system → scale-down / scale-up

Conclusions and Future work



56% of the experimetnal space tested → Not enough

Need: definition of a new experimental space

Scale-down and Scale-up

Acknowledgments

MELiSSA Pilot Plant Team

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MELiSSA

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THANK YOU.

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