Assessment of Cell Disruption Methods and Membrane Filtration for the Recovery and Purification of Lipids in Microalgae Biorefineries

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ALPO Project : recovery of lipids and polysaccharides from microalgae biomass to produce new materials

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1

High value molecules	Applications
• **Lipids**	• Biofuels
• Carbohydrates	• Food/feed
• Pigments	• By-products/co-products
• Proteins…	• **Biopolymers**

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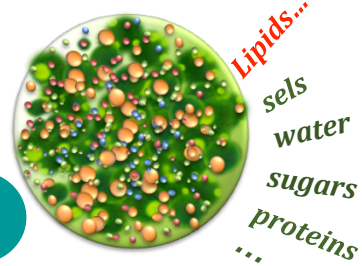
1



2

High value molecules

- **Lipids**
- Carbohydrates
- Pigments
- Proteins…

Applications

- Biofuels
- Food/feed
- By-products/co-products
- **Biopolymers**

Cell disruption methods

- **Bead milling**
- **Sonication**
- **Microwawe**
- Enzymatic treatment

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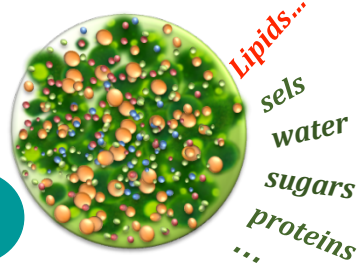

2

Cell disruption methods

To establish a procedure of **cell disruption** to extract **efficiently lipids** from biomass

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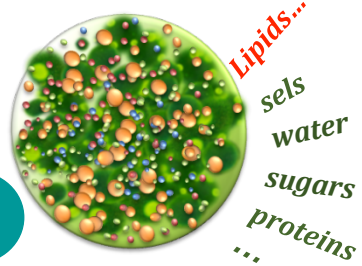

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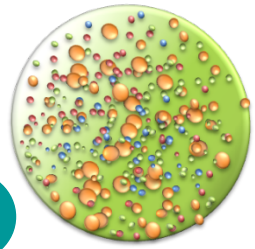

3

Fractionation/ Purification

- Solid-liquid phase separation
- Membrane filtration

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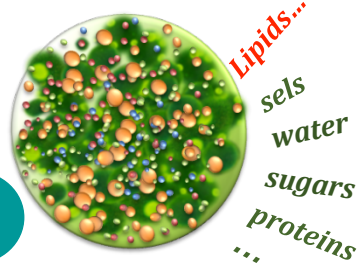

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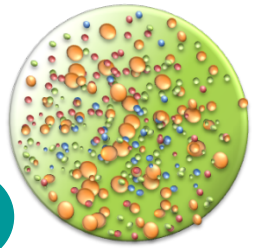

3

Fractionation/ Purification

Separation of **lipids** using technologies with a **low environmental impact**

Cell disruption methods

For all processes ➡ ***Nannocloropsis gaditana*** and ***Nannocloropsis oceanica*** at **10 g/kg**

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Cell disruption methods

For all processes → ***Nannocloropsis gaditana*** and ***Nannocloropsis oceanica*** at **10 g/kg**

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Bead mill (BM)

N. gaditana	*N. oceanica*
HFR: 150 mL/min **LFR:** 50 mL/min	**HFR:** 150 mL/ min
Ø: 0.35-0.45 mm	**Ø:** 0.35-0.45 mm **Ø:** 1.1-1.4 mm
4 cycles	**3 cycles**

Constant operating conditions:

Milling chamber filling = **80% v/v**
Rotational speed = **10 m/s**
T = **15 ± 2 °C**
Beads material: **Zr**

Cell disruption methods

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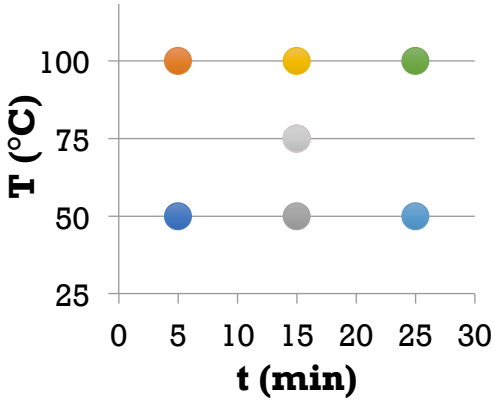
Microwave (M)

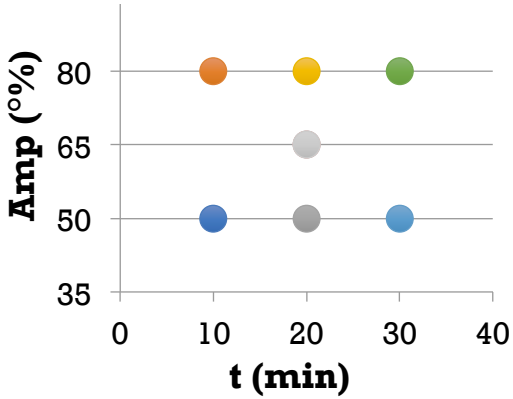
Full factorial mixed model



Constant operating conditions:

V: **20 mL**
W: **100 W**
Tf: **40 °C**
P: **0 bar**

Sonication (S)



Constant operating conditions:

V: **25 mL**;
T: **10-20 °C**;
Mode: pulse **on** **30 s**, pulse **off 5 s**

Percentage of disrupted cells

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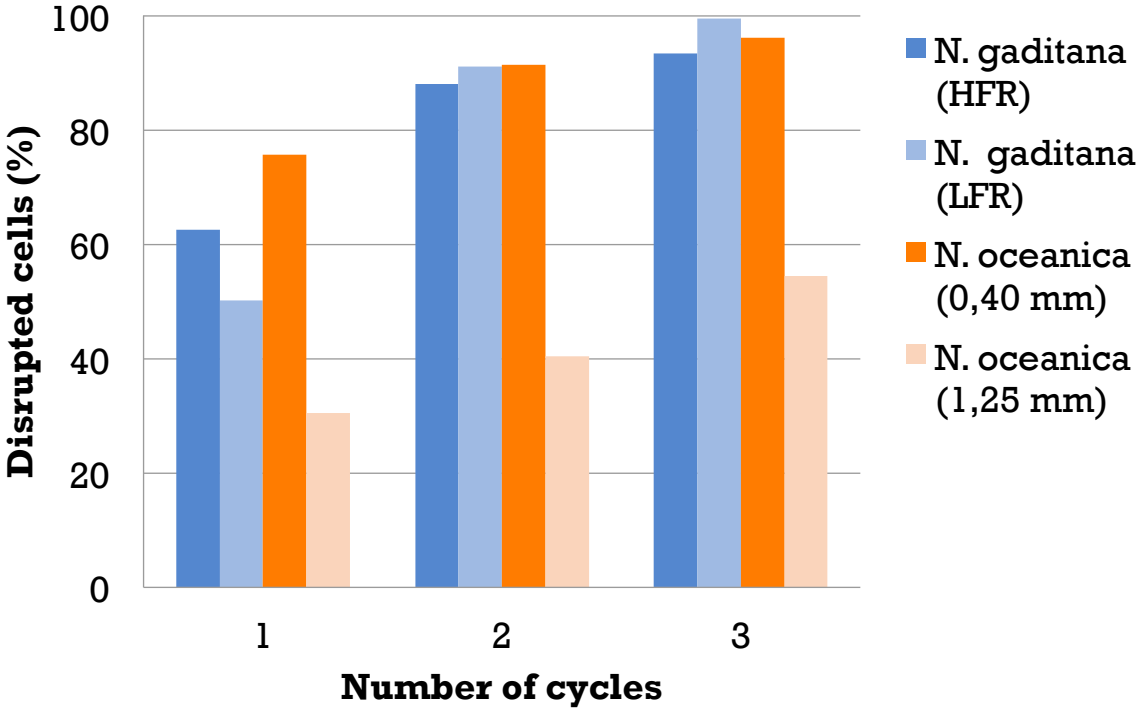

*Determined by cell counting using a Malassez counting chamber.
**HFR: High flow rate (150 mL/min); **LFR: Low flow rate (50 mL/min).

Percentage of disrupted cells

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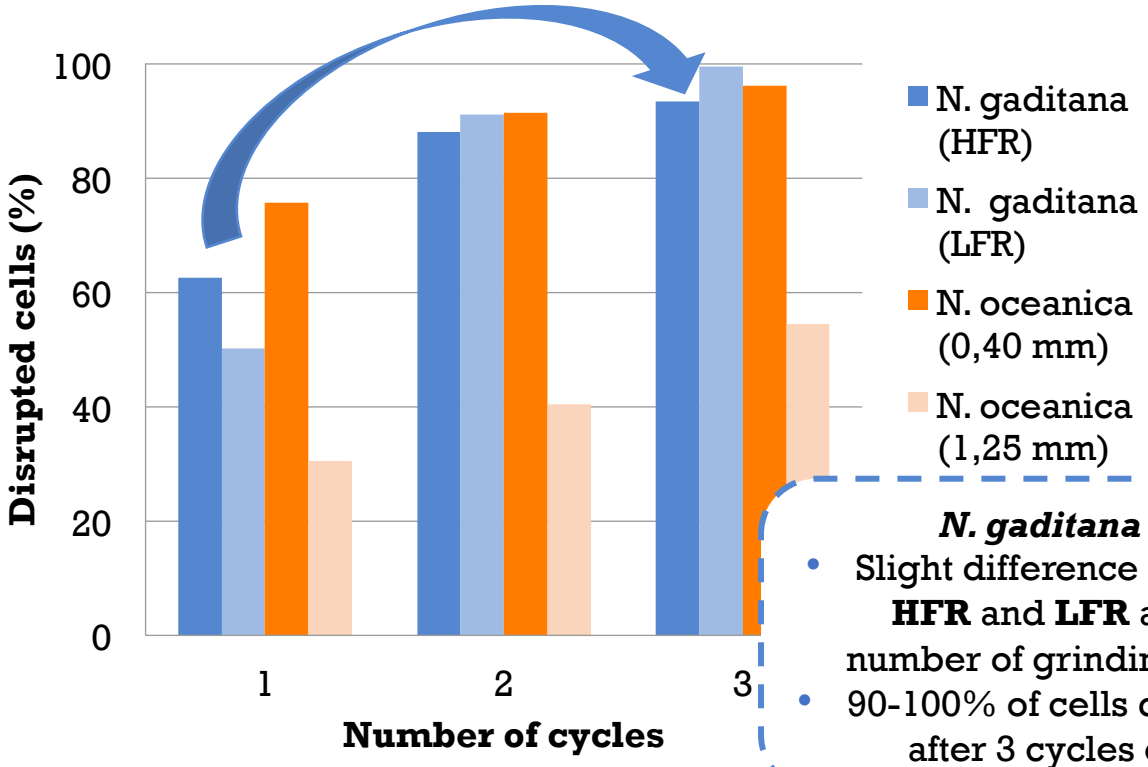
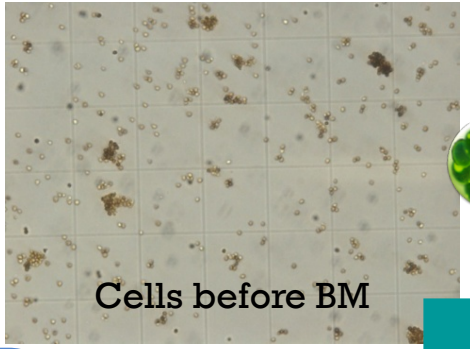
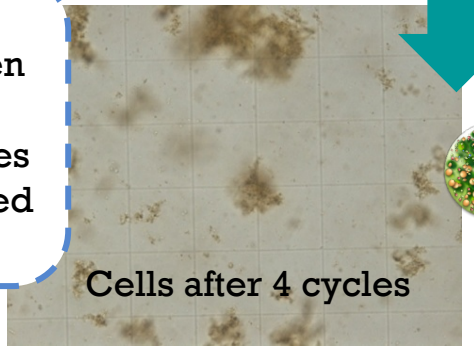


Microscopic observation of microalgae cells.
Test LFR *N.gaditana*





***N. gaditana* :**

- Slight difference between **HFR** and **LFR** at high number of grinding cycles
- 90-100% of cells disrupted after 3 cycles of BM

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****HFR**: High flow rate (150 mL/min); ****LFR**: Low flow rate (50 mL/min).

Percentage of disrupted cells

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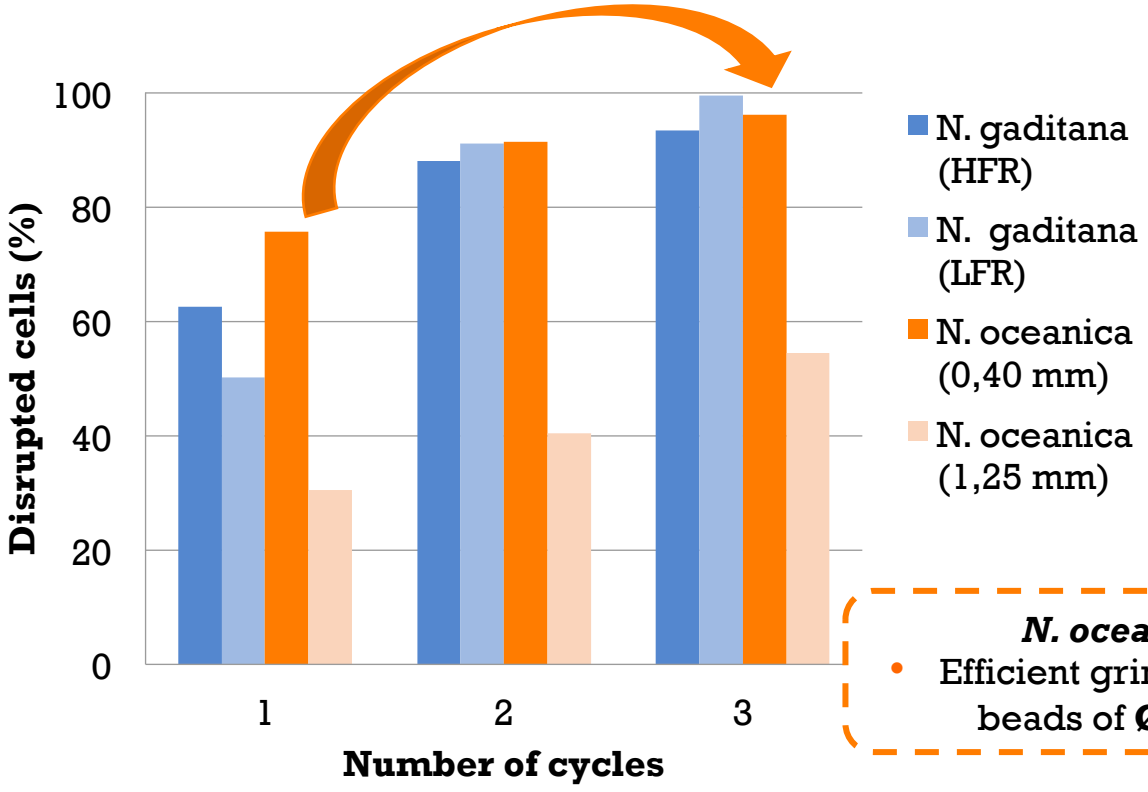

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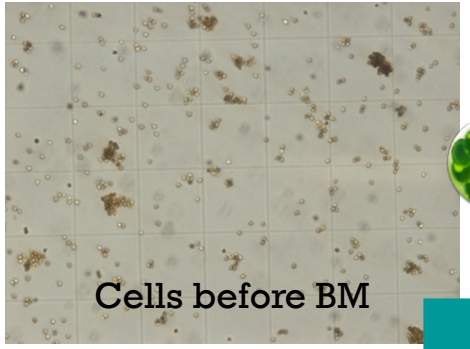

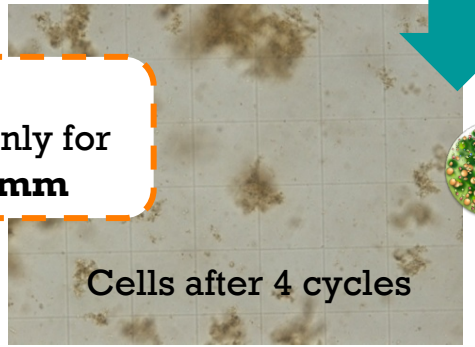

***N. oceanica* :**
- Efficient grinding only for beads of **Ø=0.40mm**

Total lipids extraction

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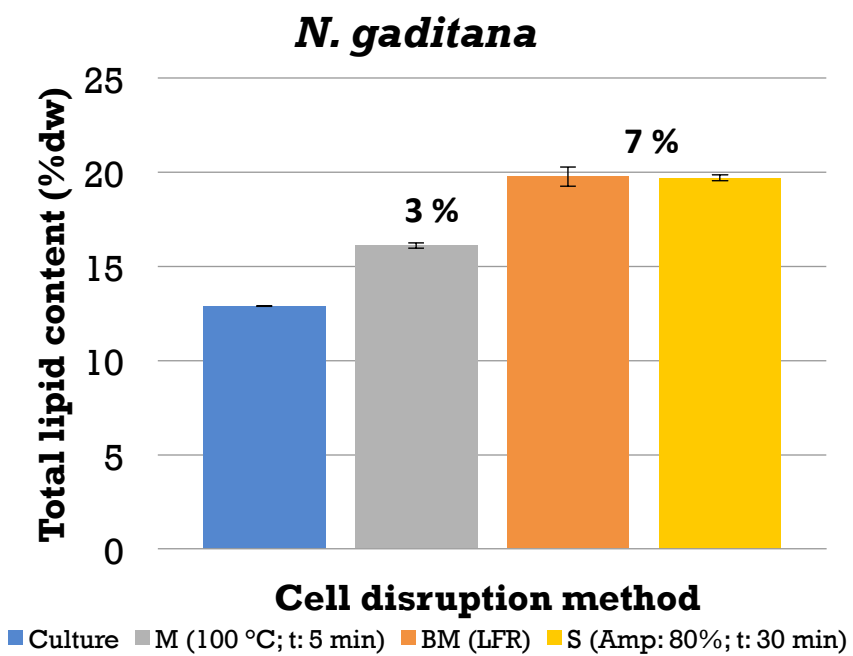

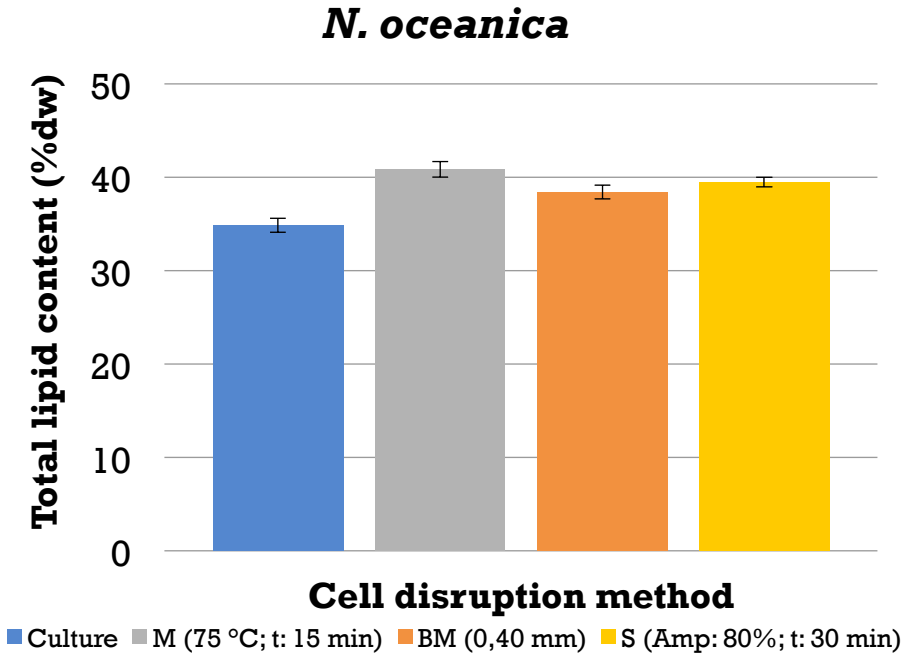

*Total lipid extraction: Obtained using the Bligh & Dyer method, with a mixture of $CHCl_3$/MeOH (2:1 v/v).

Total lipids extraction

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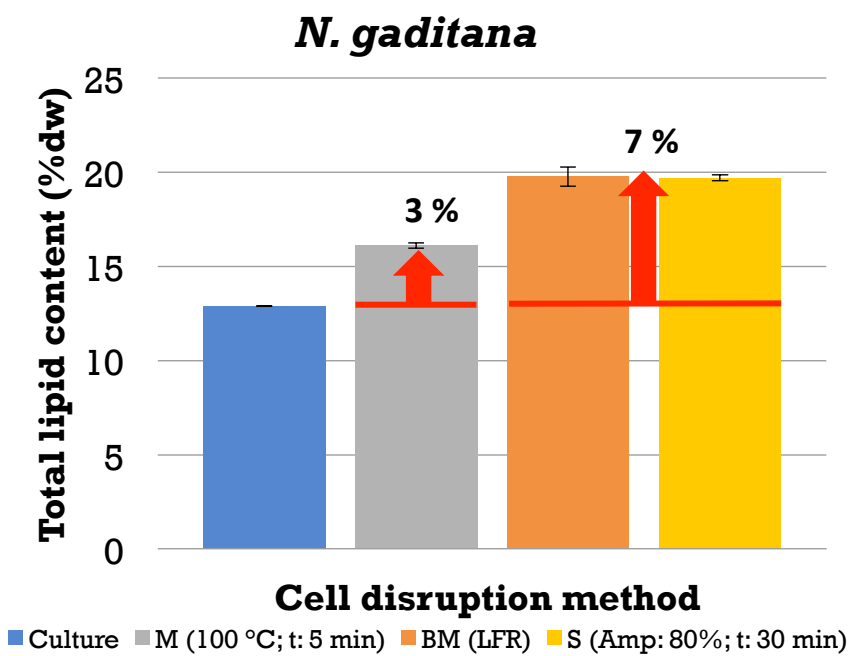

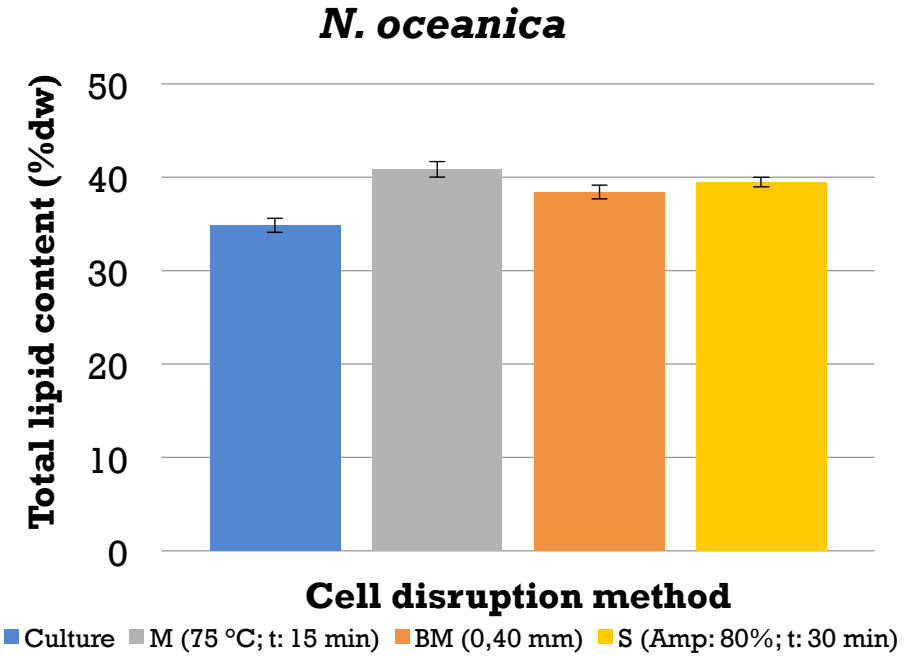

BM and S processes allow to increase in 7% the lipids recovery

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Total lipids extraction

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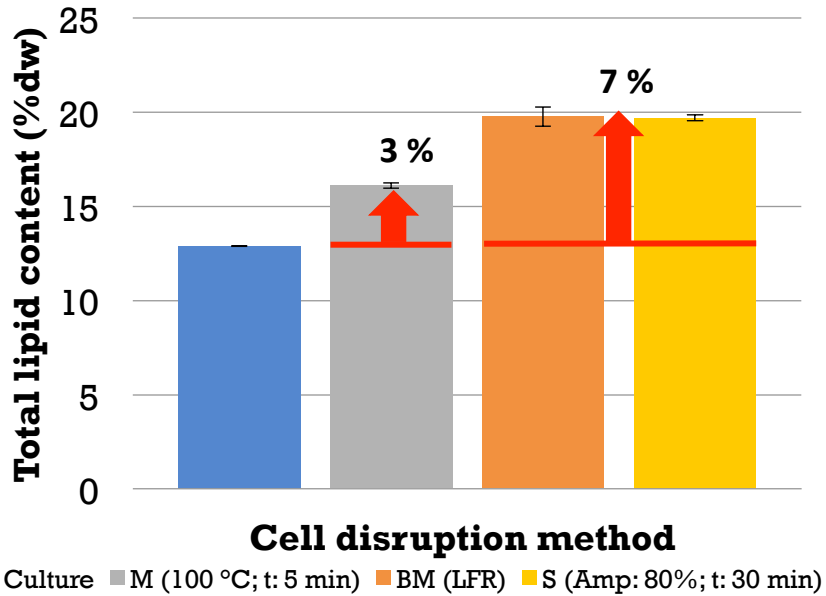
N. gaditana



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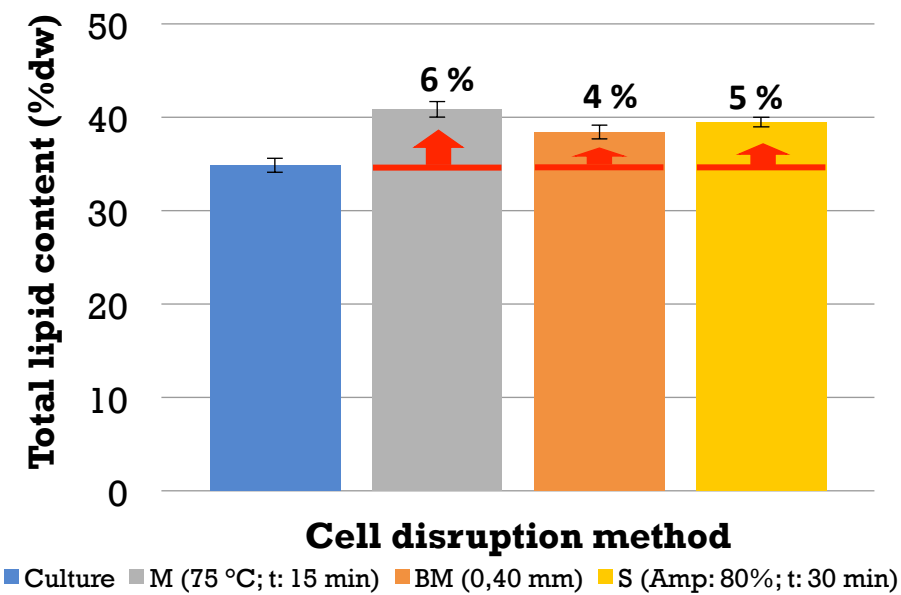

BM, S and M processes allow a slight increase in total lipid content. No difference observed in the lipids recovery when BM, S, and M

*Total lipid extraction: Obtained using the Bligh & Dyer method, with a mixture of $CHCl_3$/MeOH (2:1 v/v).

Conclusions and perspectives

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Conclusions

- **Beads material** and **diameter** must be adapted to each **microalgae**.
- **Sonication** and **bead mill** seem to be the most suitable process for lipids recovery from ***N. gaditana***.
- ***N.oceanica*** is the most interesting tested microalgae for lipids recovery, having almost **3 times higher lipids** content than the other studied strain.

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Perspectives

- Evaluate the impact of each **cell disruption** process in the **total lipids profile** by GC-MS
- Validate the **optimal conditions** of each cell disruption process : **total lipids content** and **lipid profile**.
- Set up the **enzymatic treatment**.
- **Combine cell disruption methods** to **improve lipids extraction**.
- After selecting the most suitable disruption method, **separation** and **purification** stages will be studied.

Thank you for your attention

Assessing bead milling for the recovery of lipids and sugars from microalgae

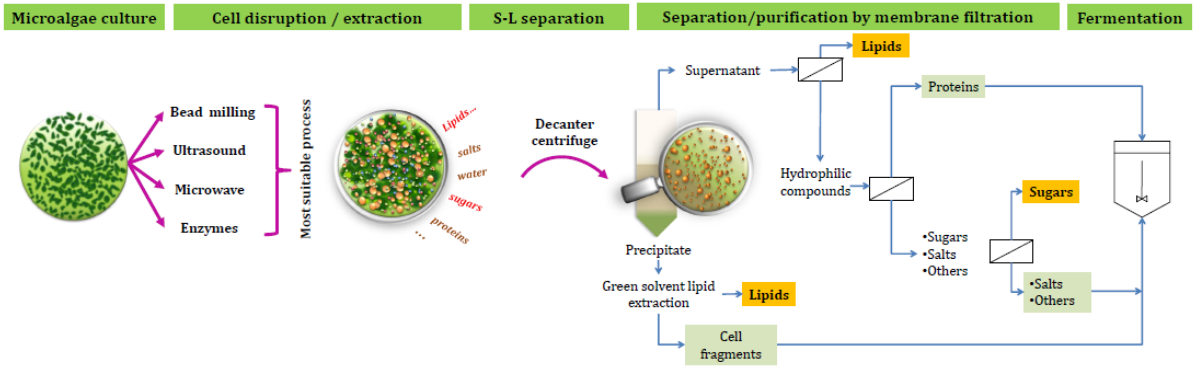
Catalina Quesada Salas, Gaëlle Willig , Erika Clavijo Rivera*, Florent Allais
Chaire ABI (Agro-Biotechnologies Industrielles), AgroParisTech, CEBB, 3 Rue des Rouges Terres, 51110 Pomacle, France
*erika.clavijorivera@agroparistech.fr

ALPO Project

- Microalgae can be used as a feedstock for high-value products. **Microalgae lipids** and **sugars** are compounds of special interest for the chemical industry because they can be used for the production of **renewable polymeric materials**.
- The biorefinery of microalgae must consider different stages for the recovery of the different components including cell disruption, extraction and purification methods, avoiding the degradation of the different molecules.

Fractionation and purification strategy

- The present work focuses initially on the **study of bead milling** (BM) **as a cell disruption method to release lipids and sugars** from the inner cell. Other methods will be also assessed: sonication, microwave and enzymatic treatment.



General approach proposed for the separation and purification of sugars and lipids