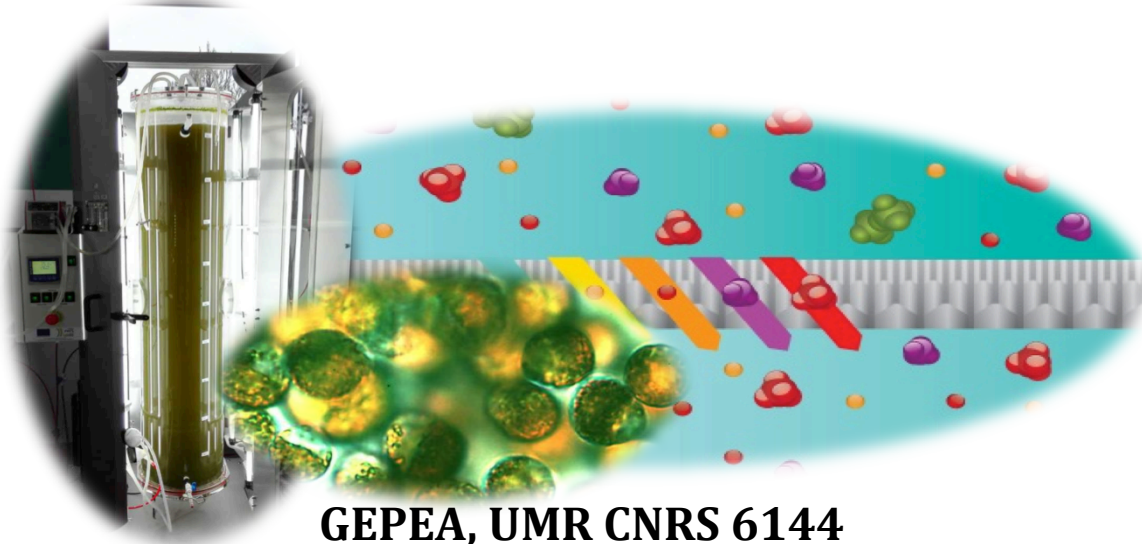


Separation of lipids from *Parachlorella kessleri* in wet processing: effect of cell destruction on the performance of the membrane fractionation process



GEPEA, UMR CNRS 6144

Shuli LIU

Laurence Lavenant

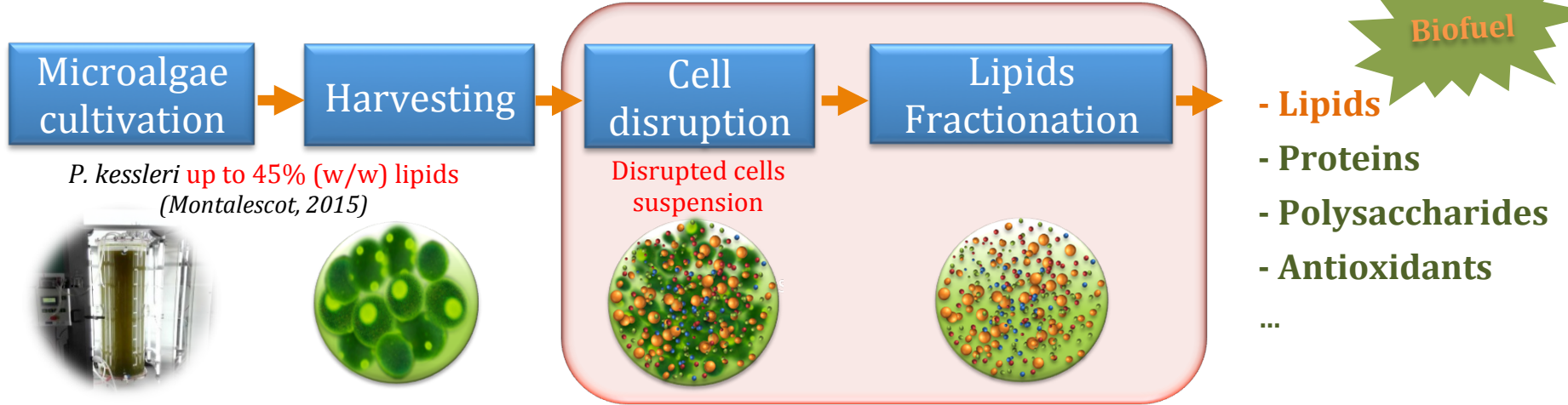
Patrick Bourseau

Matthieu Frappart

Luc Marchal

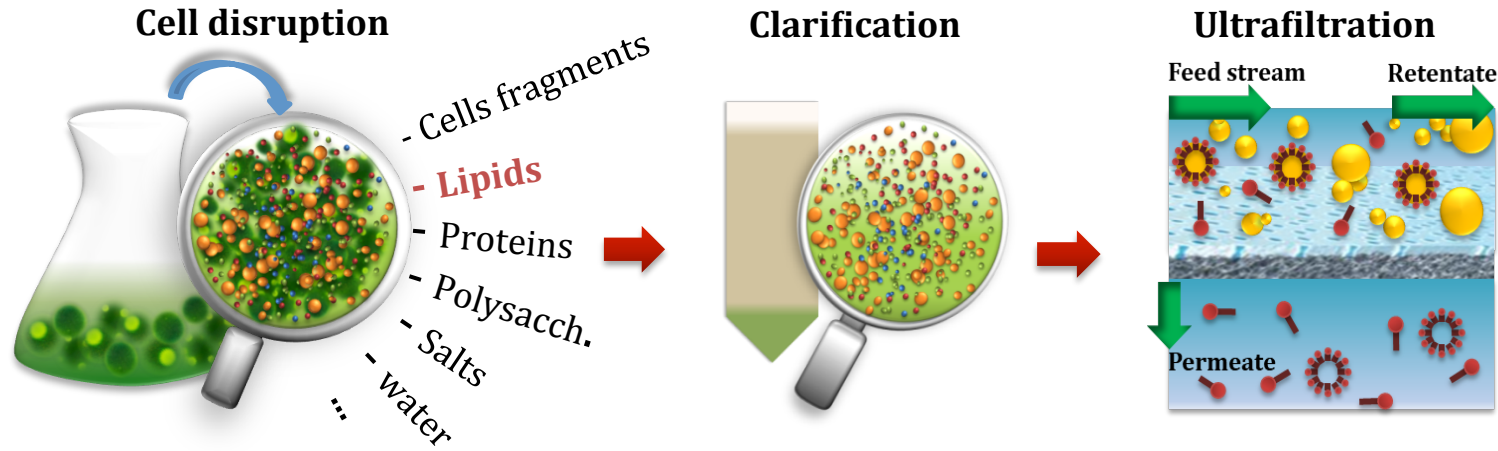
Estelle Couallier

Microalgae biorefinery

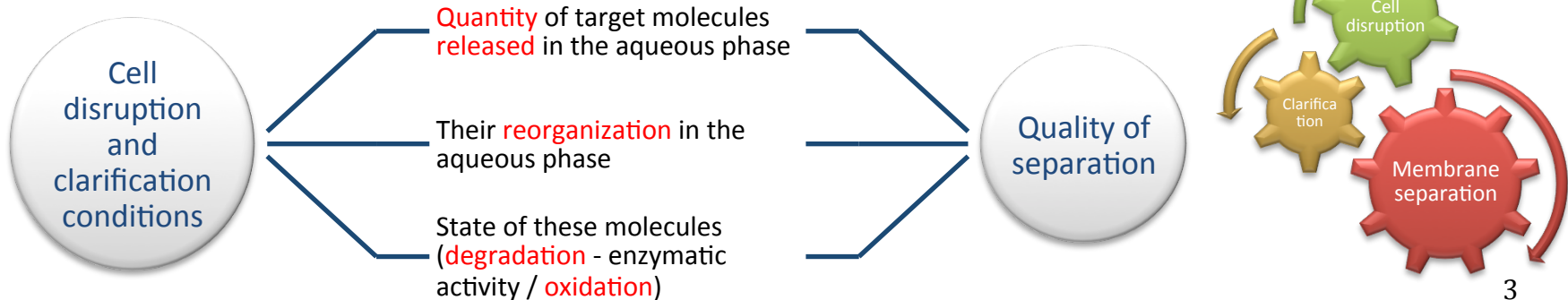


Wet way

- ✓ Reduce **energy consumption (without drying step)**
- ✓ Reduce **solvent consumption**



A new downstream processing under development...



1. To **maximize** the **release rate** of target molecules in aqueous phase
2. To **reduce** the generation of **fine particles**
3. To **reduce enzymatic activity** during grinding
4. To **avoid oxidation** during the process

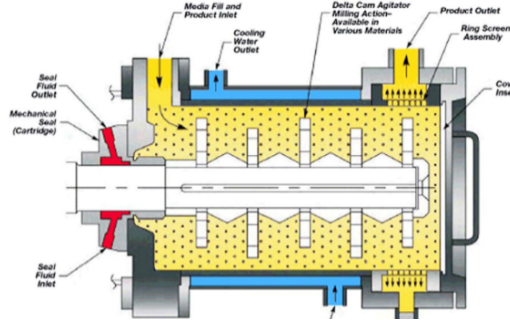
... to develop a **soft and effective** downstream processing for the lipids and proteins fractionation

Methodology



Biomass production
100L 0,5g/L DM

Lipid-rich
Parachlorella
kessleri
(5g/L DM)



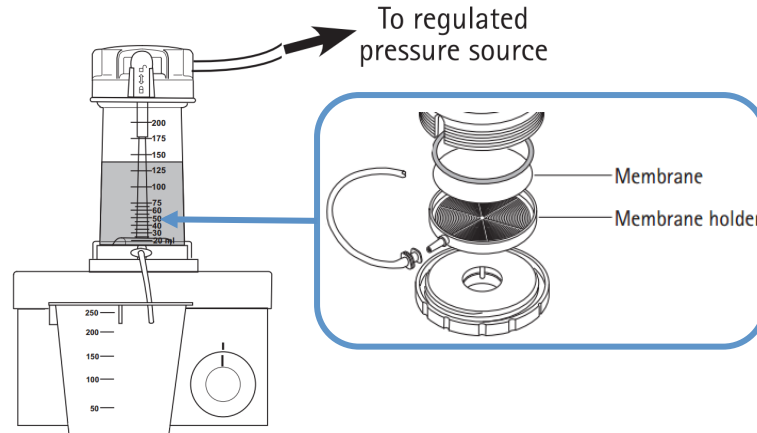
Cell disruption by bead milling
(Gunerken. 2015)

Crude biomass
(lysate)

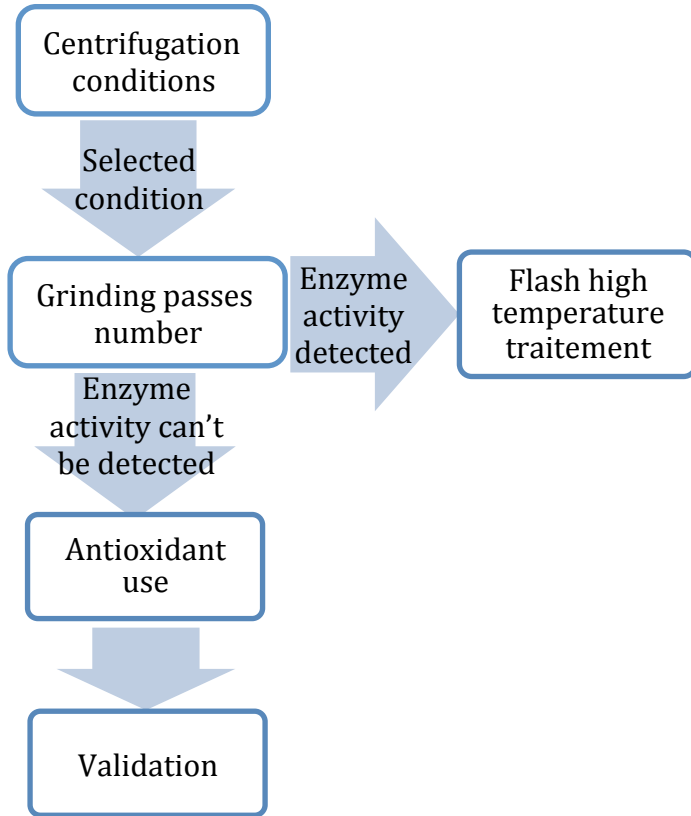


Up to 80% lipids
recovered
(Clavijo E. et al, 2017)
Supernatant

Clarification by centrifugation



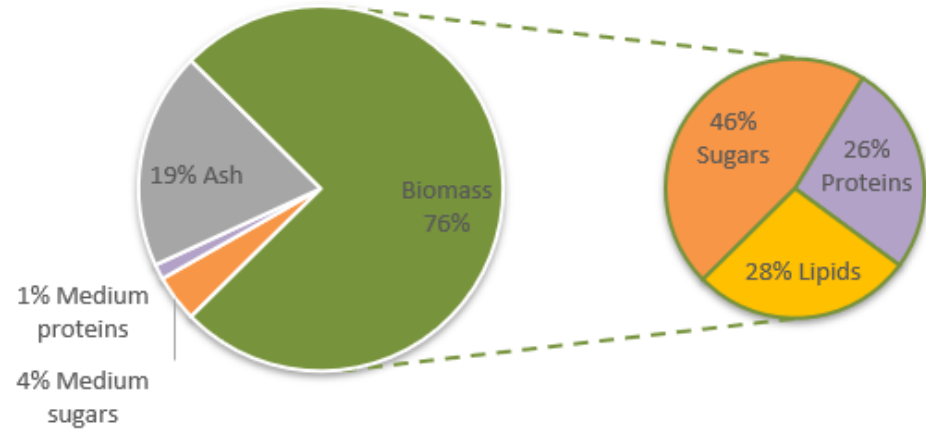
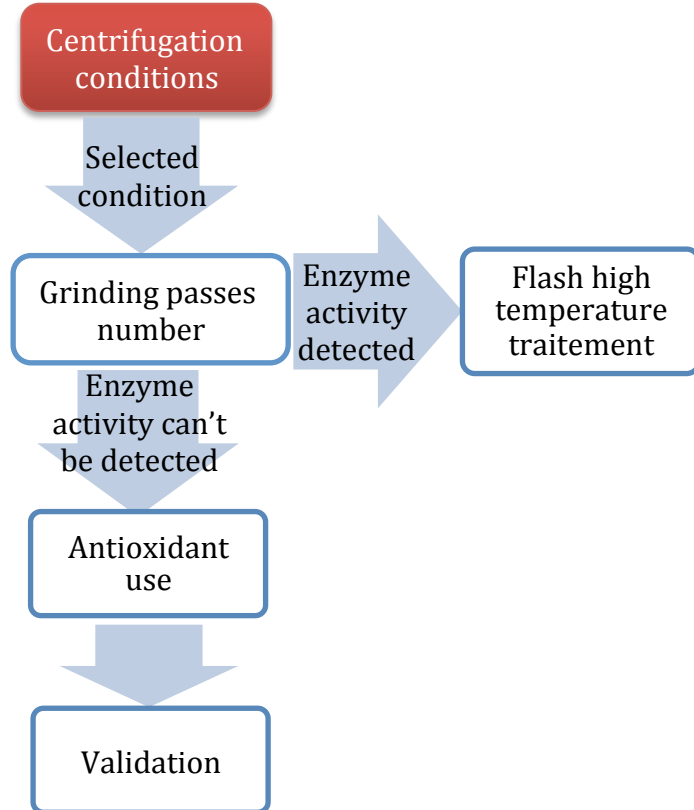
Fractionation by ultrafiltration



Parameters followed:

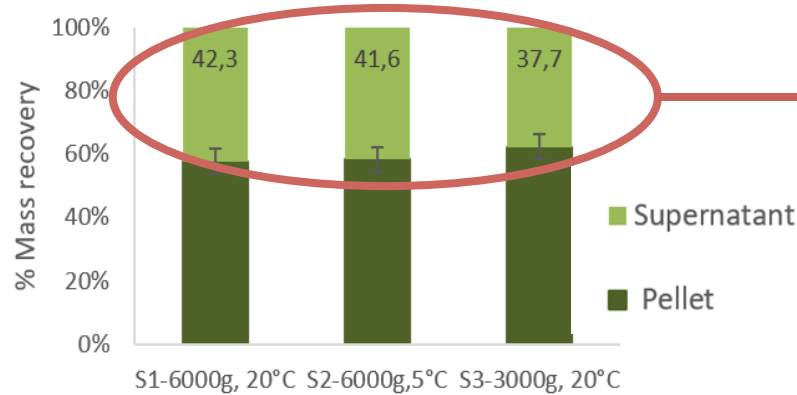
- ✓ **Quantities** of proteins, sugars, lipids **available** and **recovered**
- ✓ **Release rates** of proteins, sugars, lipids during cell disruption
- ✓ **Enzymatic activities** in lysate and in the supernatant.
- ✓ **Filtration performance** (permeate flux, membrane selectivity, retention rate etc.)

1. Centrifugation Conditions:



Bead milling	Centrifugation	Filtration
glass beads- 0,65mm	20°C, 3000g, 20min	PAN 500 kDa
chambre filling ratio 80%	20°C, 6000g, 20min	30°C at 0,3bar
flux rate 150mL/min	5°C, 6000g, 20min	VRR=3
rotation rate 8m/s		300tr/min
3 passes		

1. Centrifugation Conditions:



Operational efficiency after clarification

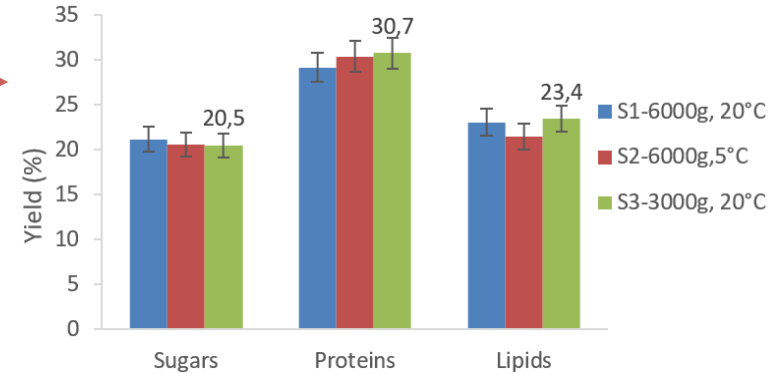
$$\begin{aligned} & \% \text{Mass recovery in supernatant} \\ &= \frac{m_{\text{supernatant}}}{m_{\text{lysate}}} \times 100 \end{aligned}$$

➤ Maximum lipid, protein recovery in the supernatant:

- 3000g, 20 °C

➤ No significant impact:

- Supernatant flux
- Selectivity



Recovery yield in the supernatant after centrifugation

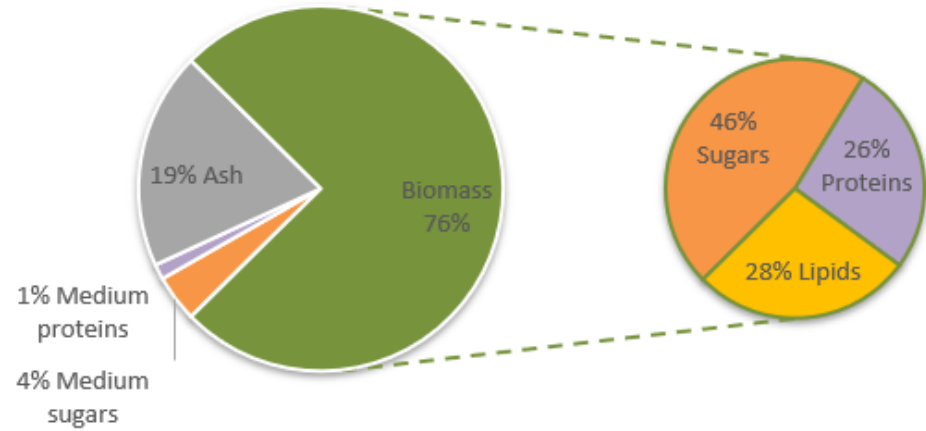
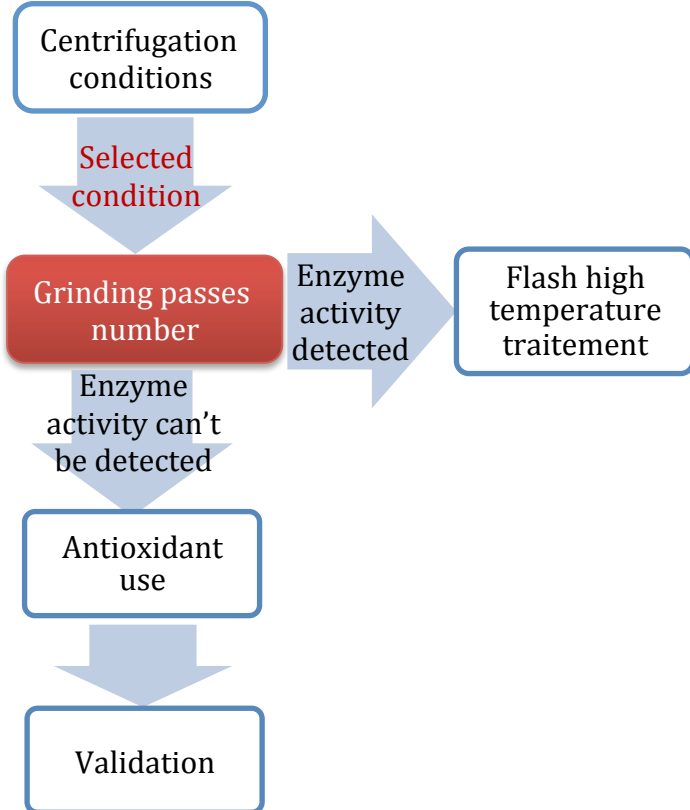
Operational efficiency

$$\begin{aligned} &= \frac{m_{\text{supernatant}}^{\text{total sugars}} \times \text{pass}}{m_{\text{biomasse+medium}}^{\text{total sugars}}} \end{aligned}$$



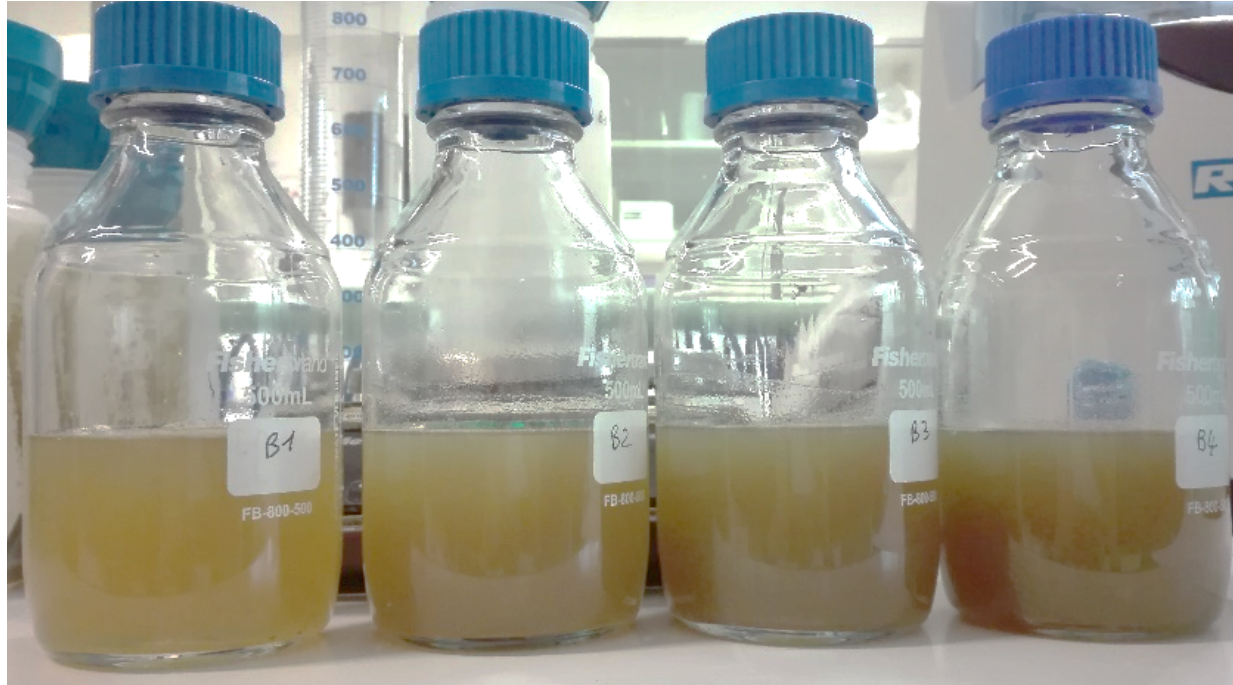
low-cost large-scale applications

2. Grinding passes number:



Bead milling	Centrifugation	Filtration
glass beads- 0,65mm	20°C, 3000g, 20min	PAN 500 kDa
chambre filling ratio 80%		30°C at 0,3bar
flux rate 150mL/min		VRR=3
rotation rate 8m/s		300tr/min
1,2, 3, 4 passes		

2. Grinding passes number:



1 pass

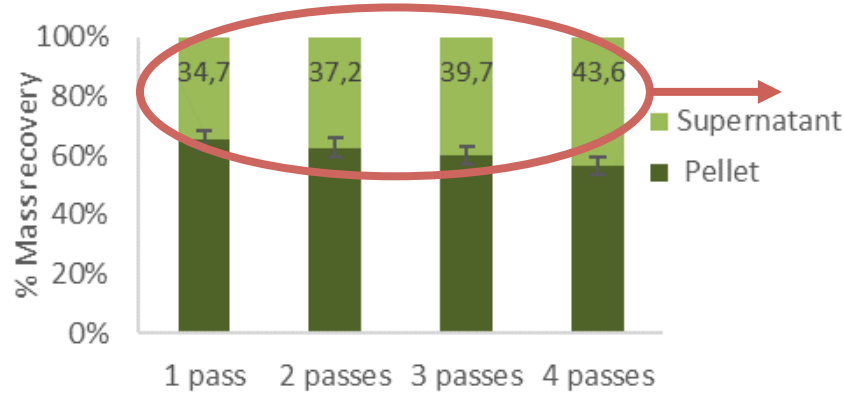
2 passes

3 passes

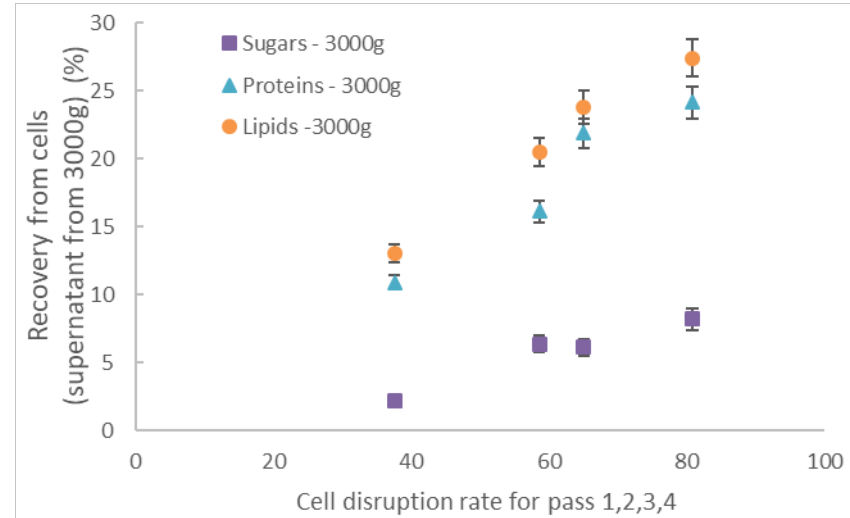
4 passes

Clarified supernatants from each pass of lysate

2. Grinding passes number:



Mass distribution after clarification



Compounds recovered in the supernatant from the cells

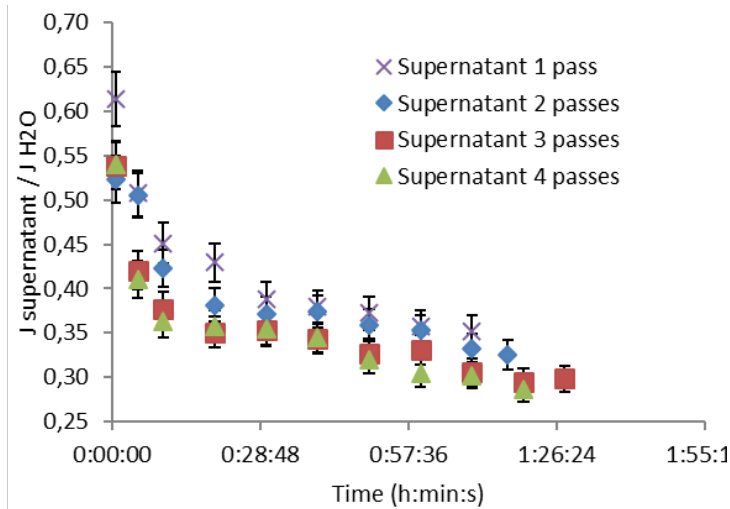
After the 2nd pass:

- Extra recovered proteins are mostly insoluble proteins (suspended particles)
- Extra recovered lipids are cell membranes lipids (phospholipids/glycolipids) or aggregates

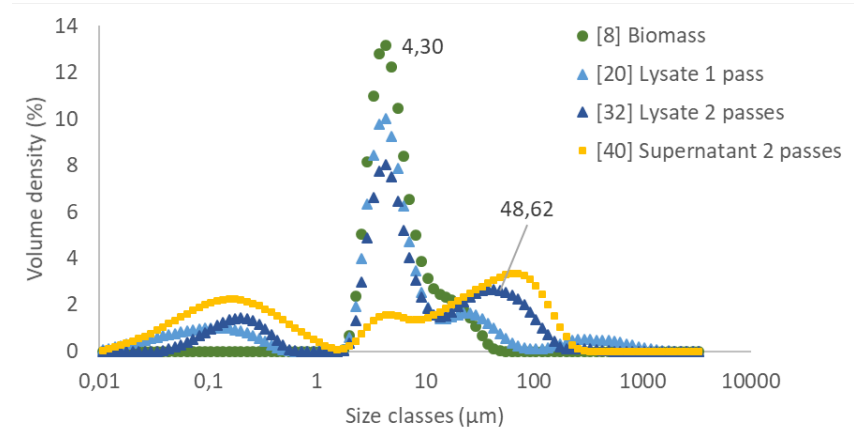
% Recovery rate from cell

$$= \frac{m_{\text{supernatant}}^{\text{total sugars}} - m_{\text{medium}}^{\text{sugars}}}{m_{\text{biomass+medium}}^{\text{total sugars}} - m_{\text{medium}}^{\text{sugars}}} \times 100$$

2. Grinding passes number:



Evaluation of normalized supernatant flux during concentration (VRR3)

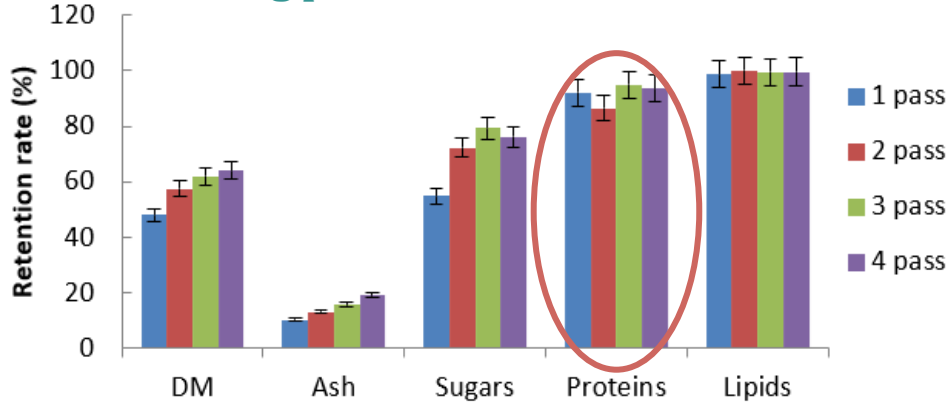


Volume distribution of the particles in the biomass, lysate and supernatant

- Permeation fluxes: $J_{1\text{pass}} > J_{2\text{passes}} > J_{3\text{passes}} = J_{4\text{ passes}}$
- ↗ Generation of fine particles → recovered in the supernatant to be filtered: higher risk of membrane fouling
- Increase passes number → aggregates generation

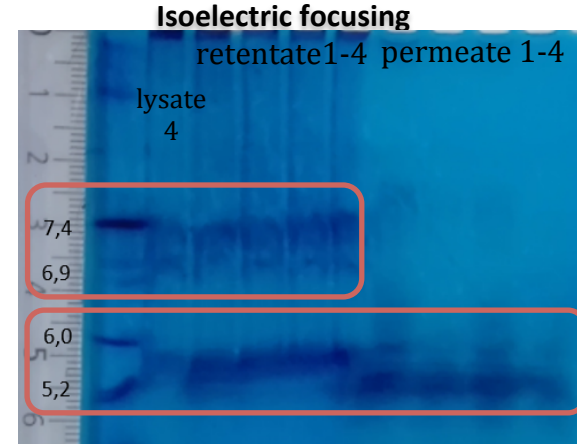
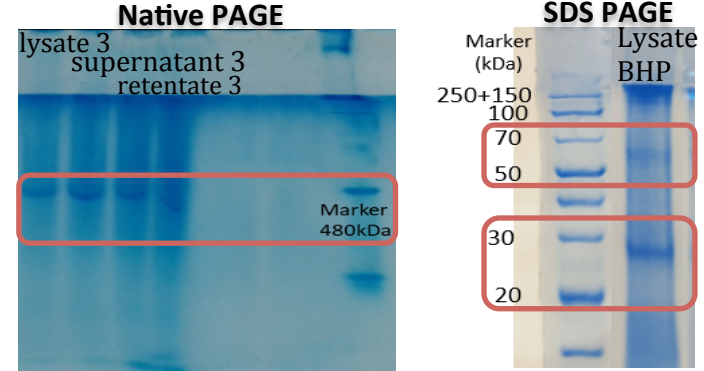
Results and Discussion

2. Grinding passes number:



Retention rate after concentration in VRR3

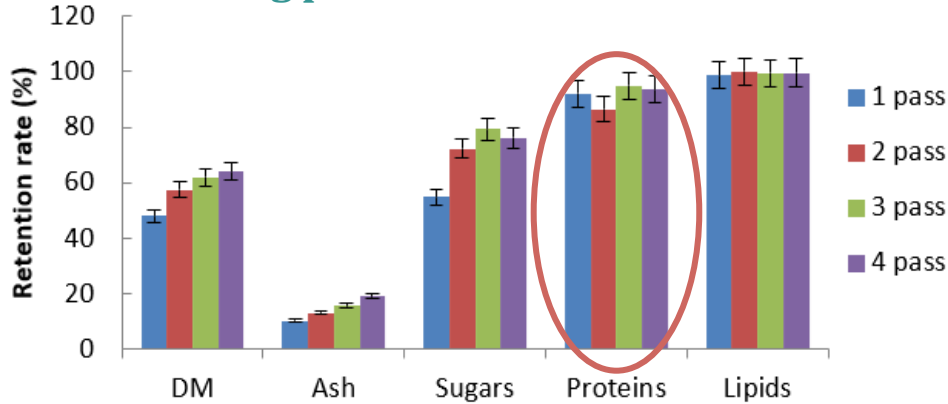
- Total lipids retention, good separation between lipids and salts
- Recommended condition : 2 passes
- Future improvement to separate lipids and proteins:
 - ✓ change of physicochemical conditions → keep membrane preselected
 - ✓ change the membrane → higher lipids fouling resistance



Protein characterization results (Electrophoresis)
(Pr. at pi 6,0-5,2 not retained)

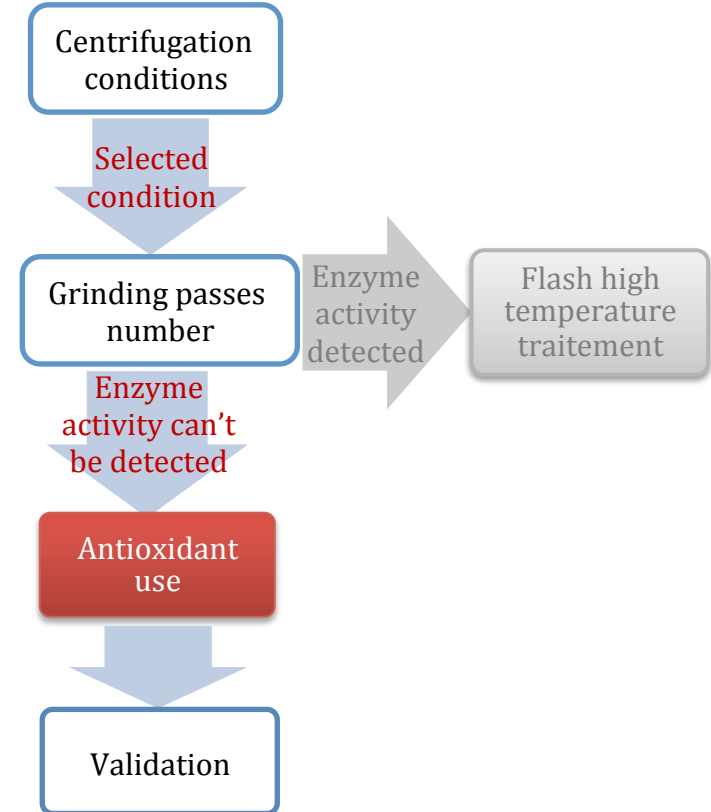
Results and Discussion

2. Grinding passes number:

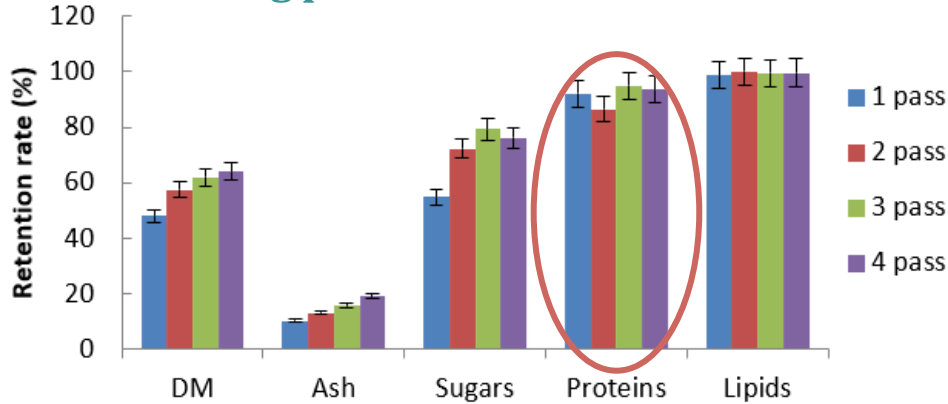


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2. Grinding passes number:

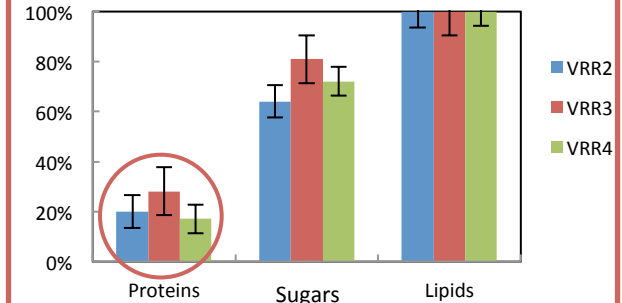


Retention rate after concentration in VRR3

- Total lipids retention, good separation between lipids and salts
- Recommended condition : 2 passes
- Future improvement to separate lipids and proteins:
 - ✓ change of physicochemical conditions → keep membrane preselected
 - ✓ change the membrane → higher lipids fouling resistance

Fractionation of lipids/ proteins seems possible.

Retention rate - real mixture of ground N.G.
(protein antioxidant used during disruption)



Results of experience done with another microalga NG.

Conclusions:

- ✦ Centrifugation conditions has few impact for the filtration process
3000g, 20°C → low-cost large-scale applications
- ✦ ↗ passes number :
 - ↗ fines particles generation
 - ↗ insoluble proteins recovery
 - ↗ polar lipids recovery or aggregates

Future works:

- ✦ Change physicochemical conditions for a better proteins/lipids separation
- ✦ Understanding the reorganization of the compositions after destruction and their interactions (L/L; L/M)
- ✦ Highlight the consequences of these interactions to improve the filtration performances (optimize filtration conditions)

Thank you for your
attention

Acknowledgements:

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Presented by:
Shuli LIU