FROM RESEARCH TO INDUSTRY

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Innovation for Industry

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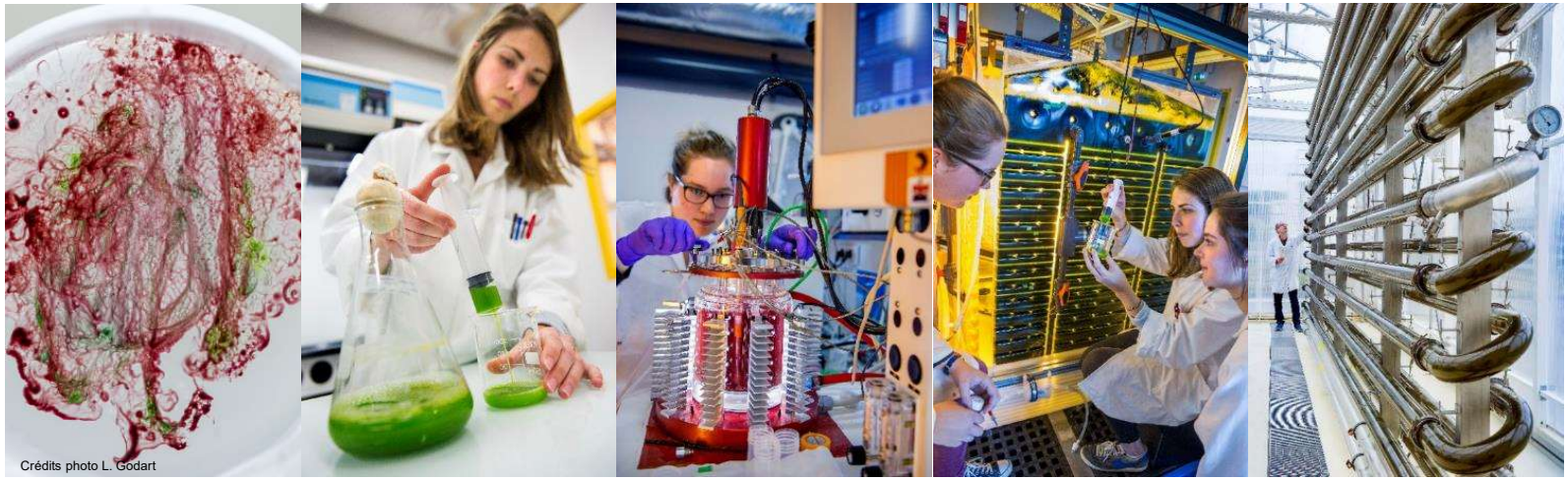

CHLORELLA VULGARIS HIGH-GLUCOSE SYRUP AS CARBON FEEDSTOCK FOR PHA-PRODUCING BACTERIA

Pablo Alvarez[1], Compadre A[1]., Six A[1]., Fleury G[1]., Dubreuil C[1]., Lainé C[2]., Lemechko P[3]., Bruzaud S[2]., Sassi J.F[1].

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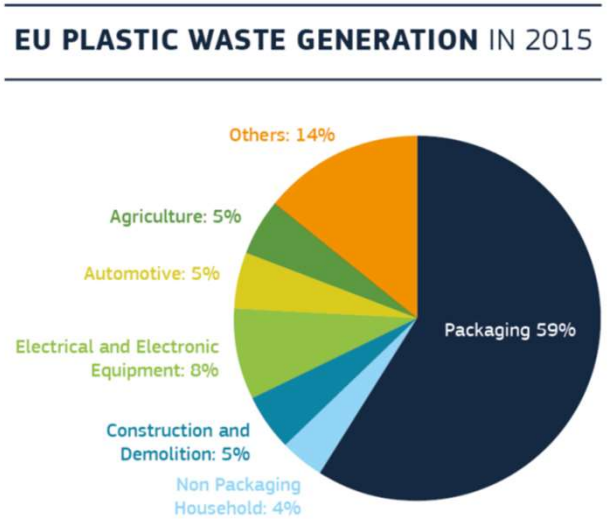
TOO MUCH PLASTIC

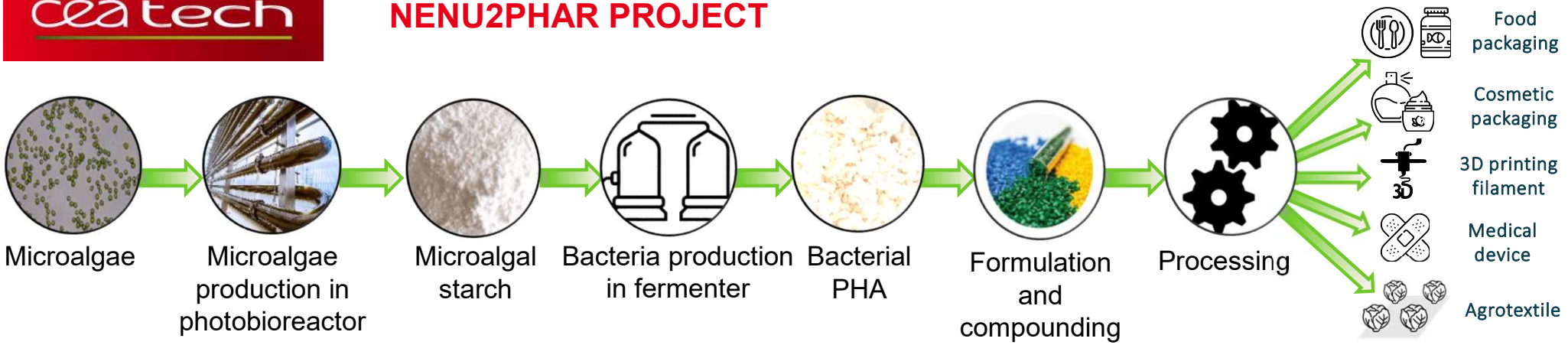
- **Global production of plastics:**
  322 million tonnes (2015)
- **EU plastic waste:**
  25.8 million tonnes / year



We need to reduce plastic consumption but also we need more **BIOPLASTICS**

Conventional plastics (Fossil-based, Non-biodegradable)		**Bioplastics** (Bio-based, Bio-degradable)
Polypropylene (PP)		Starch blends
Polyethylene (PE)		Polylactic acid (PLA)
Polyethylene terephthalate (PET)		Polybutylene succinate (PBS)
		Polyhydroxalcanoate (PHA)

NENU2PHAR PROJECT



nenuPHAr

For a sustainable and European value chain of PHA-based materials for high-volume consumer products

BBI JU contribution: €4.9 million

Duration: September 2020 – February 2024

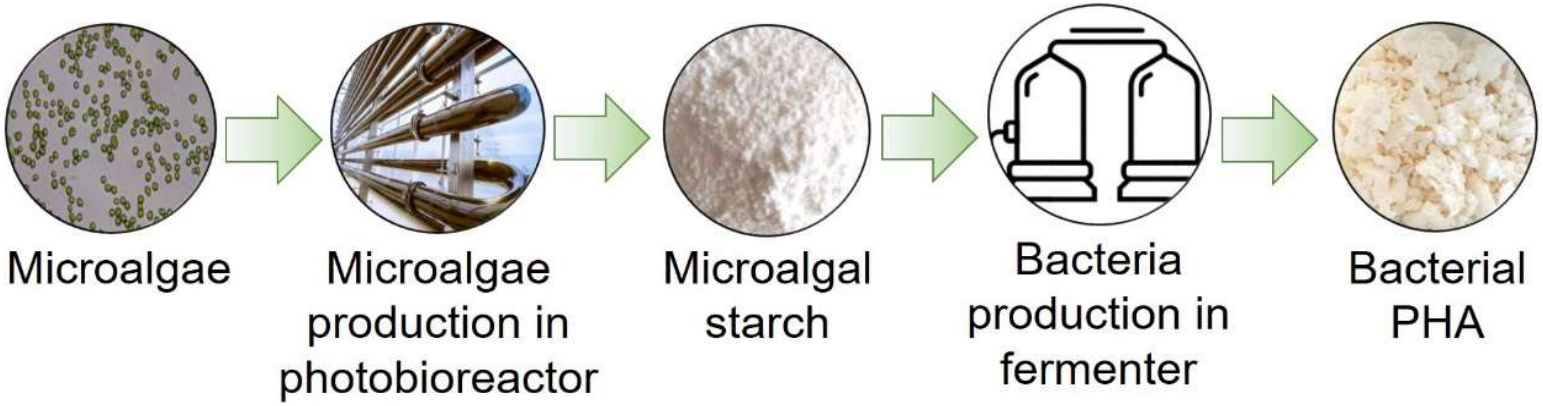
4 RTOs
1 Academic
1 Innovation Cluster

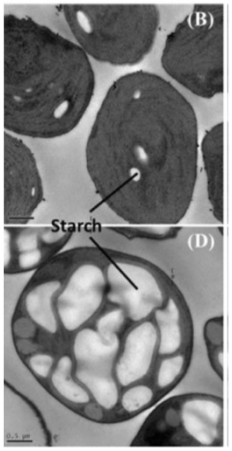
6 SMEs

4 Large Companies

This project has received funding from the Bio Based Industries Joint Undertaking (BBI-JU) under grant agreement No 887474. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.

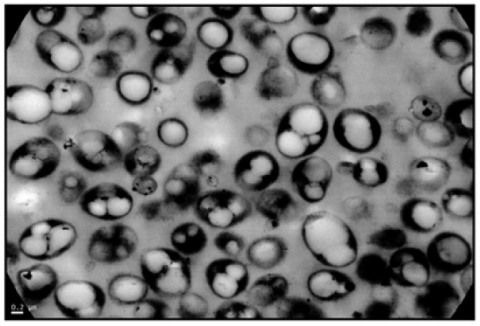
KEY CONCEPT: STARCH FROM MICROALGAE





- **Starch granules in nutrient-limited *Chlorella vulgaris***

Cheng et al., (2017)
Improving carbohydrate and starch accumulation in Chlorella sp. AE10 by a novel two-stage process with cell dilution.

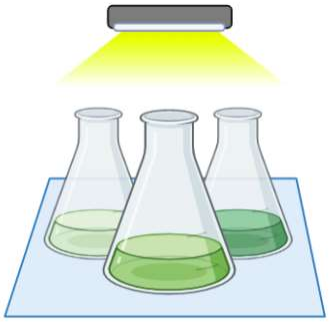


PHA granules in nutrient-limited *Cupravidis necator*

Nygaard et al., (2021)
PHA granule formation and degradation by Cupriavidus necator under different nutritional conditions

PROCESS AT LAB-SCALE IN ERLENMEYER

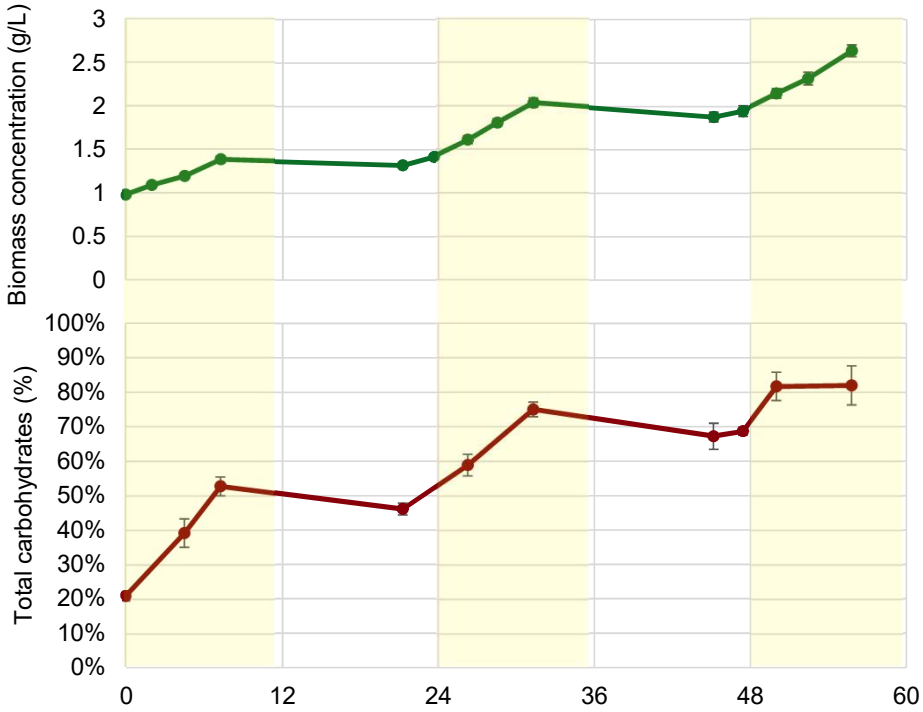
***C. vulgaris* CCALA924**



Erlenmeyers
in culture chamber

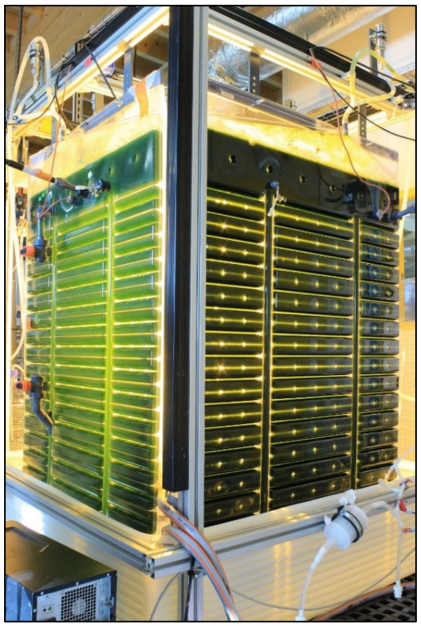
200µE/m2/s
11h/13h (Day/Night)
25°C, 2%CO2,150rpm

NO_3- depleted medium



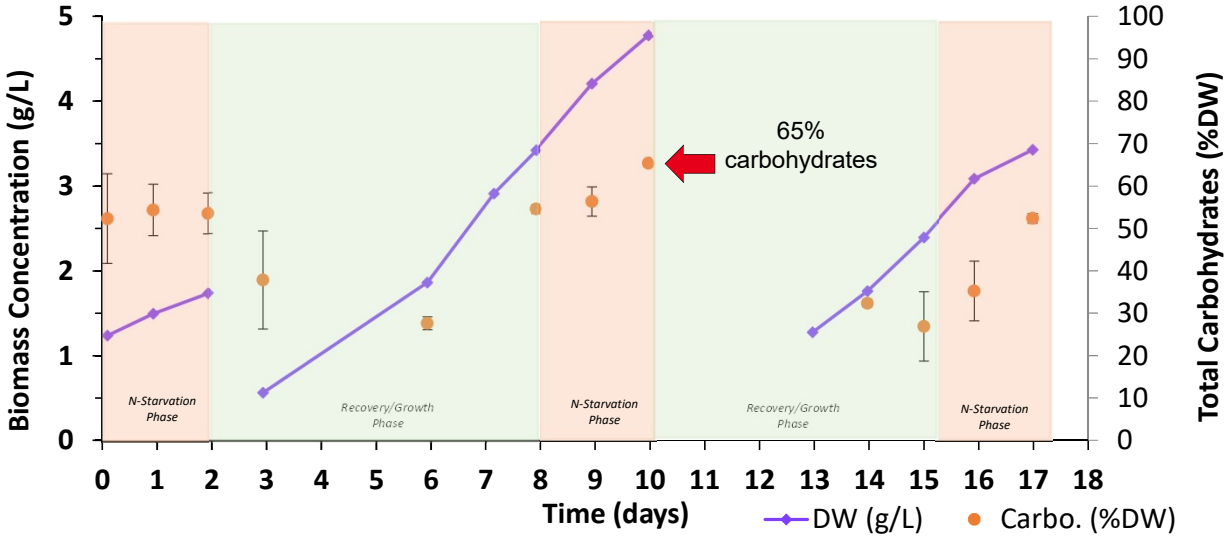
80% carbohydrates

PROCESS AT LAB-SCALE IN PHOTOBIOREACTOR



25L Flat panel airlift photobioreactor

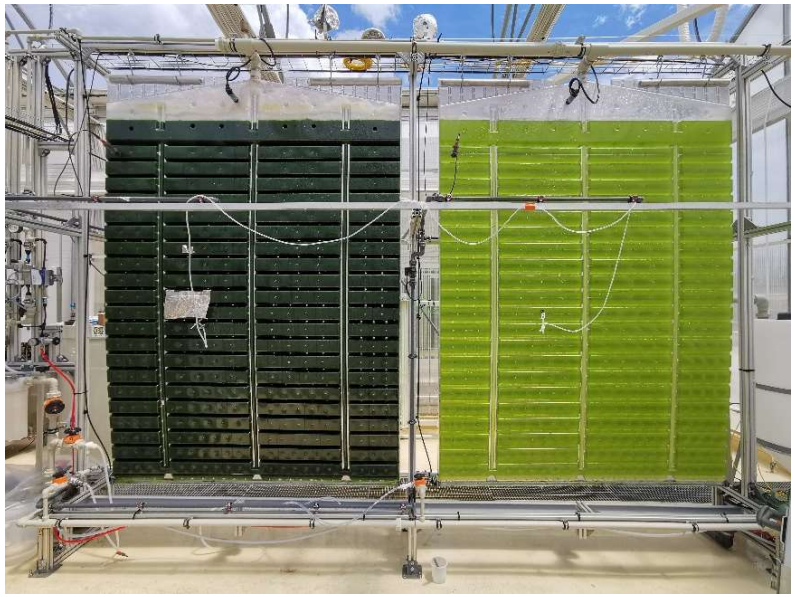
240µE/m2/s
20h/4h (Day/Night)
25°C, 2%CO2,



Chlorella vulgaris, A PROMISING FEEDSTOCK FOR STARCH-BASED BIOPLASTICS

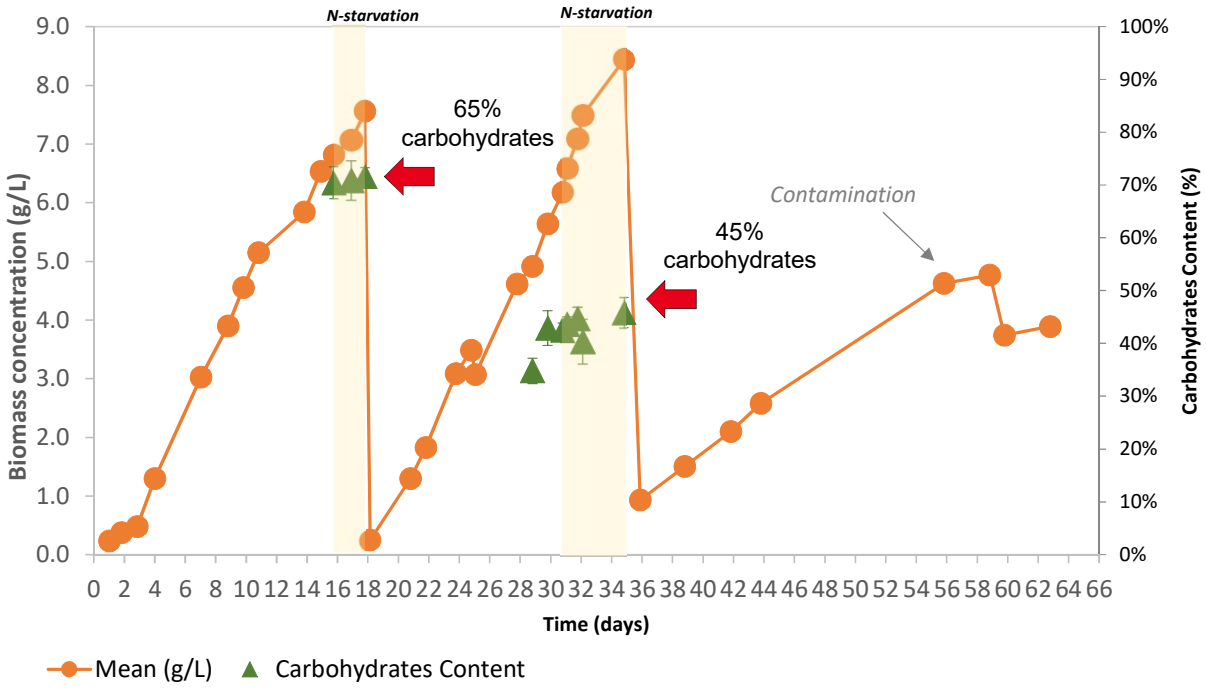
Poster: Compadre et al. (2021)

PROCESS AT SEMI-INDUSTRIAL SCALE IN PHOTOBIOREACTOR



180L Flat panel airlift photobioreactor

25°C, 2%CO2,

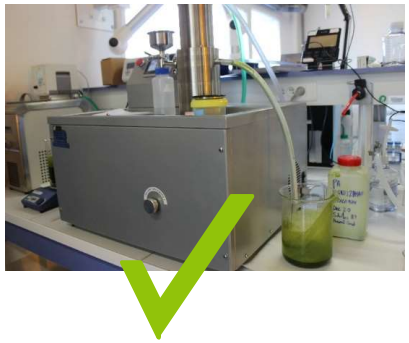


DOWNSTREAM PROCESSING: MECHANICAL *C. VULGARIS* DISRUPTION

Ultrasound (US)

Pulsed Electric Field (PEF)

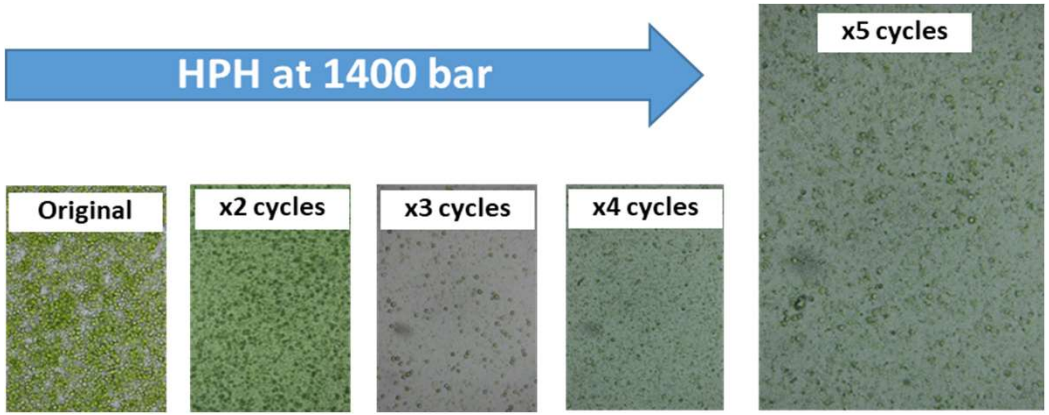
High pressure homogeneization (HPH)



HPH works but too much energy.

Can we optimize this?

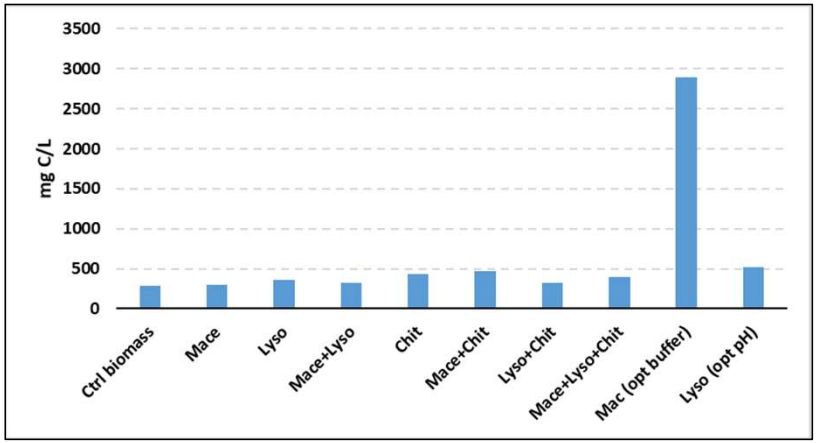
Enzymatical treatment

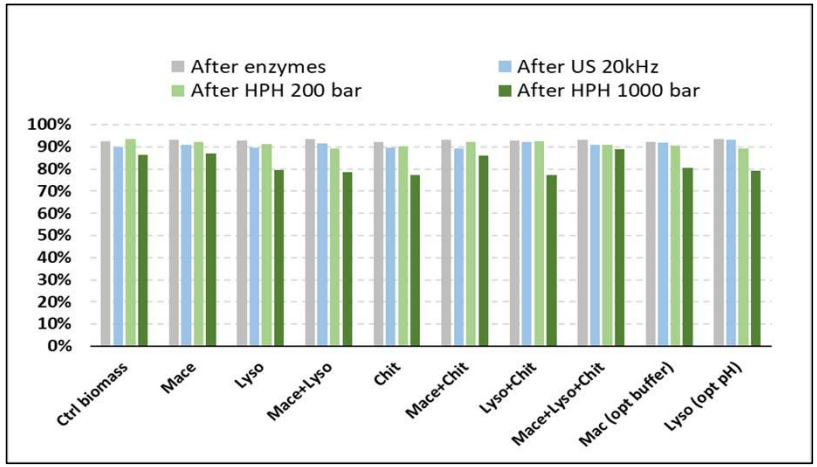


DOWNSTREAM PROCESSING:
ENZYMATIC PRE-TREATMENT FOR *C. VULGARIS* DISRUPTION

3 enzymes to degrade *C. vulgaris* cell wall	Macerocyme (Pectinase)	Lysozyme	Chitinase

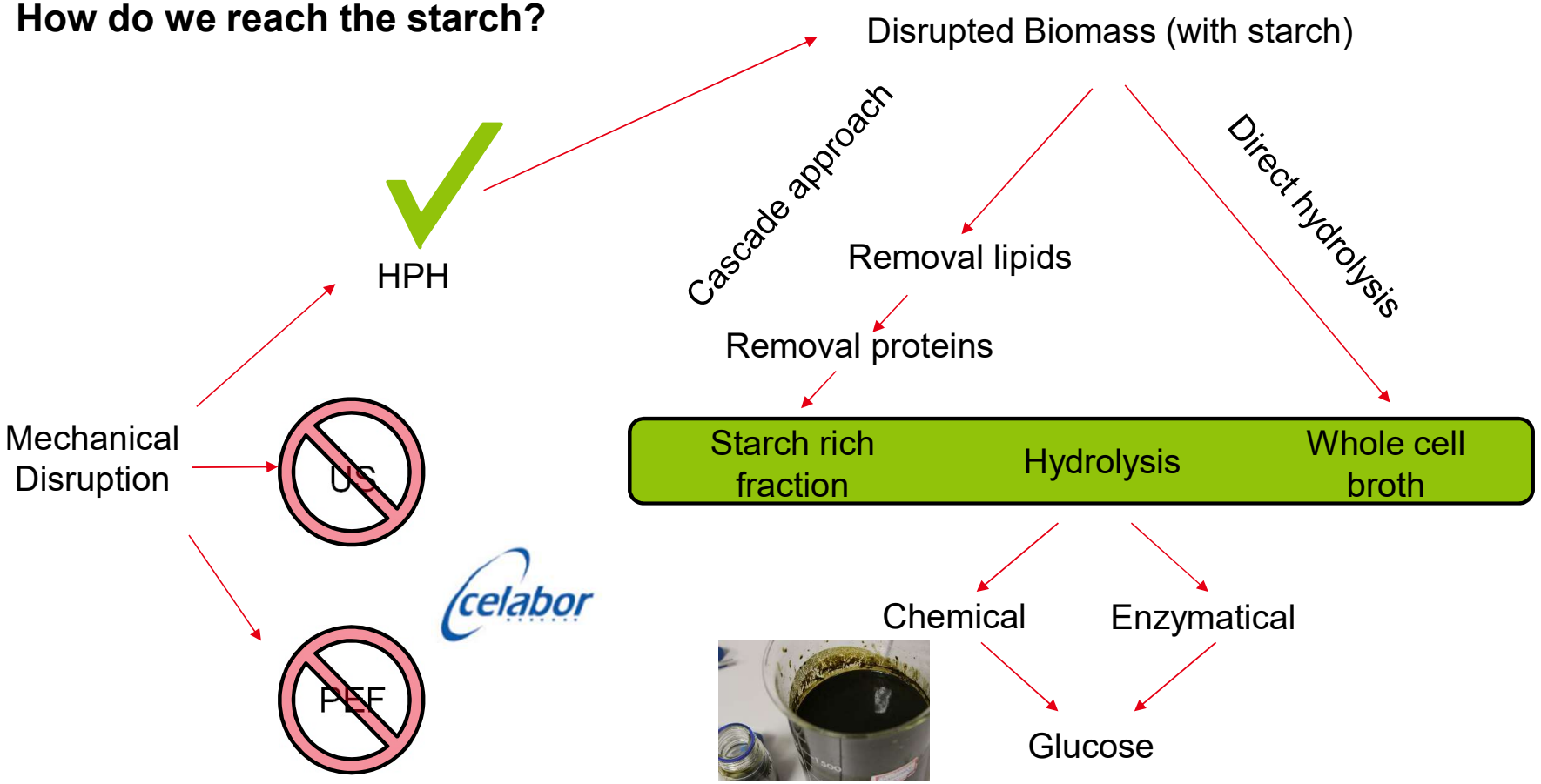
Experiment	Factor		
	Macerocyme	Lysozyme	Chitinase
1: Ctrl Biomass	0	0	0
2: Mace	1	0	0
3: Lyso	0	1	0
4: Mace+Lyso	1	1	0
5: Chit	0	0	1
6: Mace+Chit	1	0	1
7: Lyso+Chit	0	1	1
8: Mace+Lyso+Chit	1	1	1
Extra			
*9: Mace (opt pH)	1	0	0
*10 (opt pH)	0	1	0



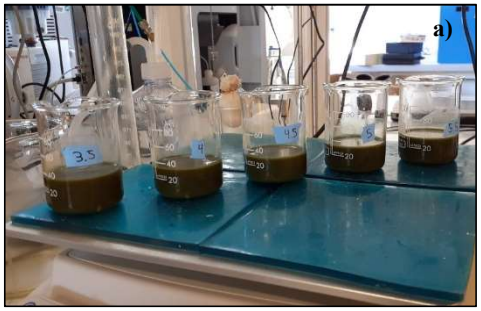


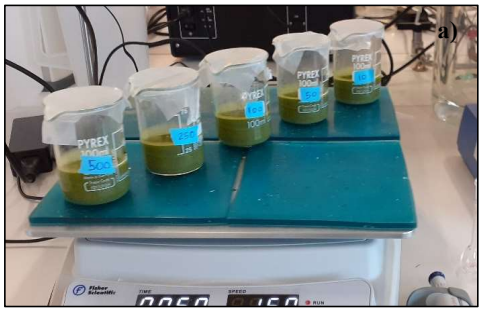
DOWNSTREAM PROCESSING:
MECHANICAL *C. VULGARIS* DISRUPTION + ENZYMATIC HYDROLYSIS OF STARCH

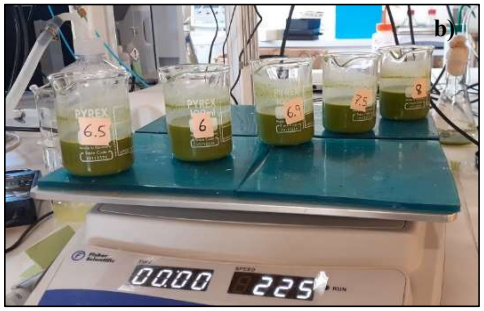
How do we reach the starch?



DOWNSTREAM PROCESSING: ENZYMATIC HYDROLYSIS OF CELL BROTH

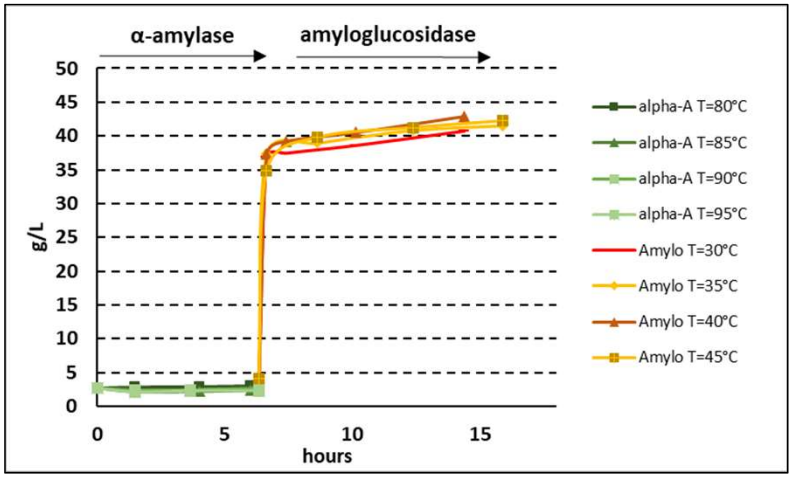


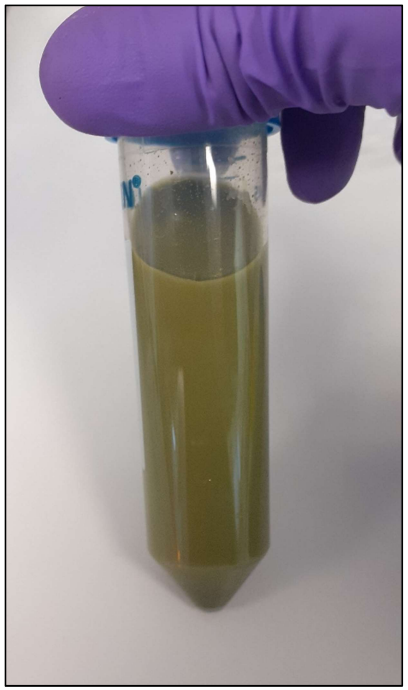




SIGMA-ALDRICH:
α-amylase → A6180
amyloglucosidase →A7095

Glucose release



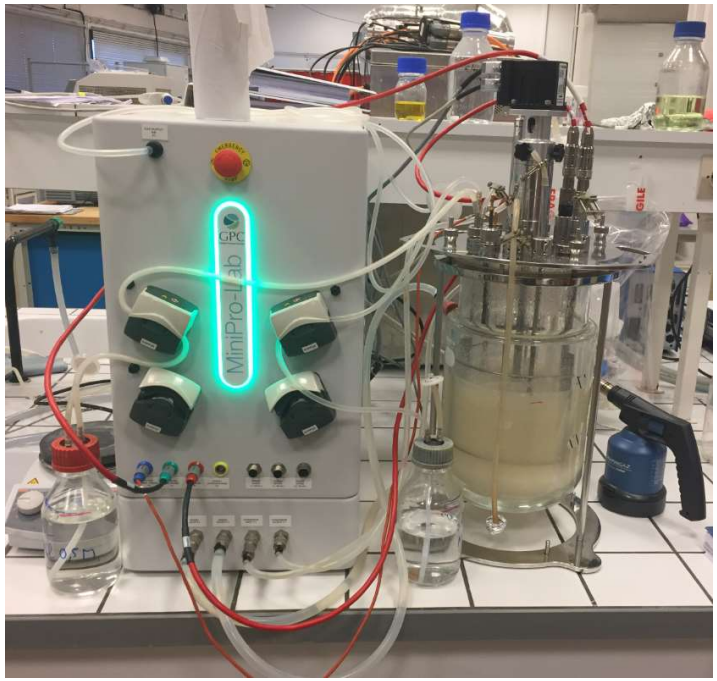


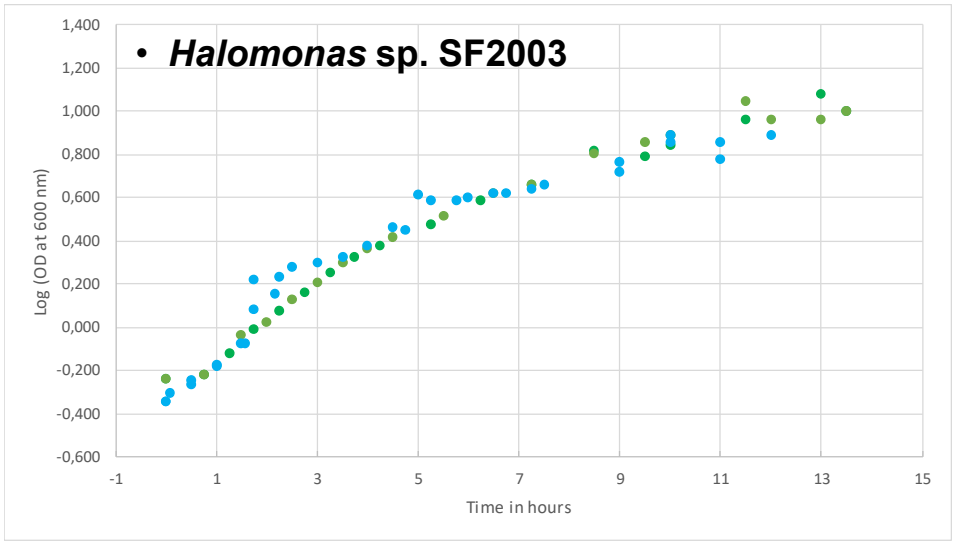
Novozymes:
1) α-amylase → Liquozyme SC4X
2) Amyloglucosidase → Spirizyme Fuel HS

MICROALGAL GLUCOSE AS FERMENTATION SUBSTRATE

- ***Halomonas* sp. SF2003**

Control = commercial glucose
Test = Hydrolyzed algal supernatant





MICROALGAL GLUCOSE AS FERMENTATION SUBSTRATE
CELL BROTH CLARIFICATION

Lab extraction in chloroform

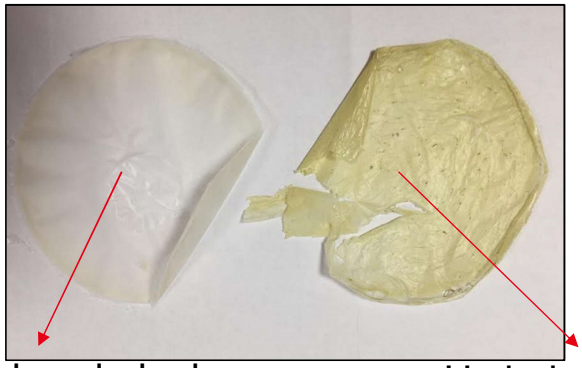
Water phase

Biomass residue

Organic phase containing PHB

Slow drying at room temperature in Petri dish

PHA extracted

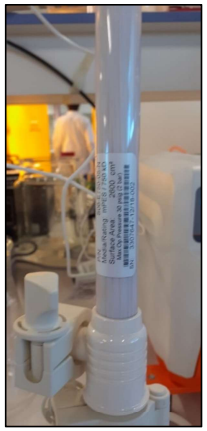


Hydrolyzed algal supernatant as substrate

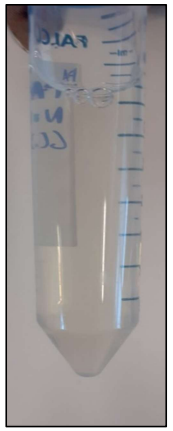
Hydrolyzed algal broth as substrate

Tangential Filtration

7.5 g/L glucose

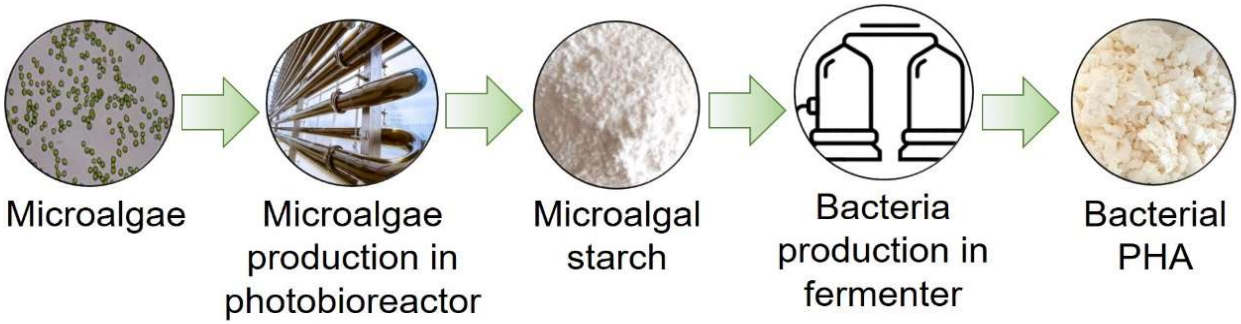


750 KDa Tangential filtration

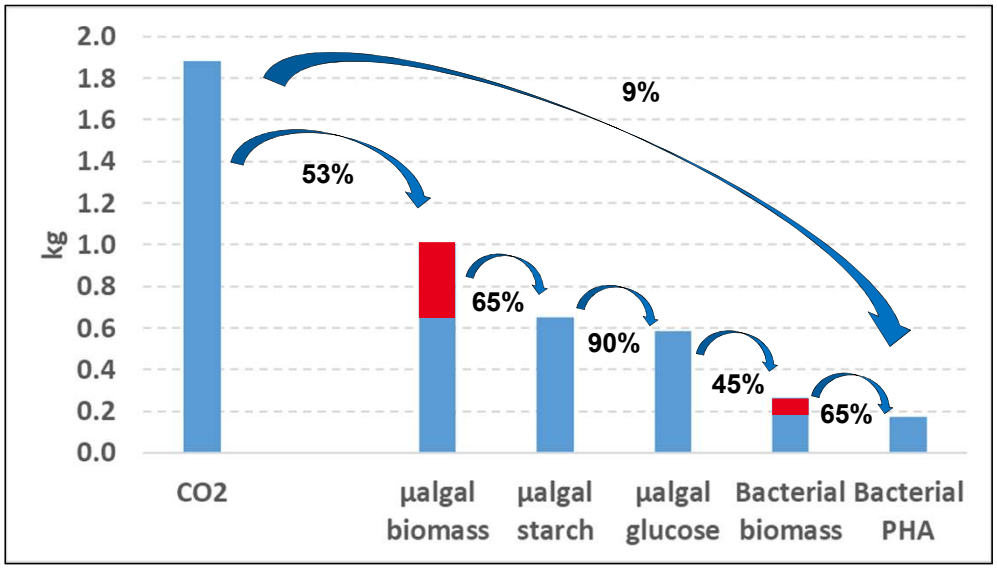


5.9 g/L glucose

FROM ALGAE TO PHA: MASS BALANCE



Conversion rates



1kg of PHA → 5,8 kg of µalgae

1kg of PHA → 11 kg of CO2

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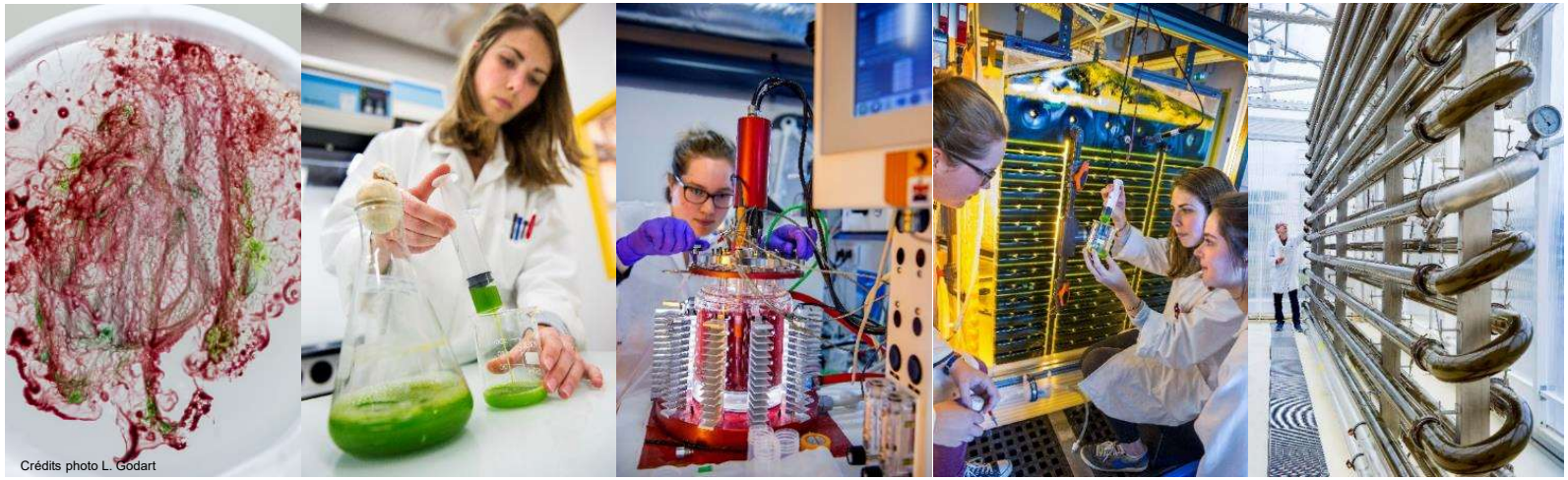

Crédits photo L. Godart

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