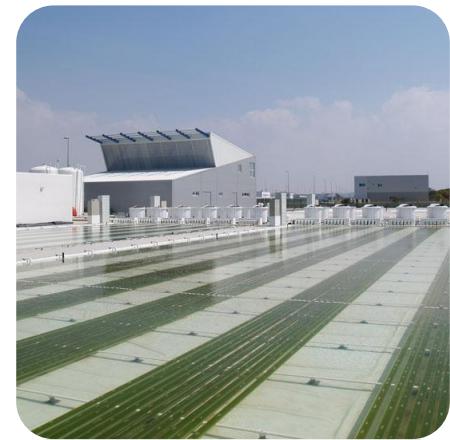
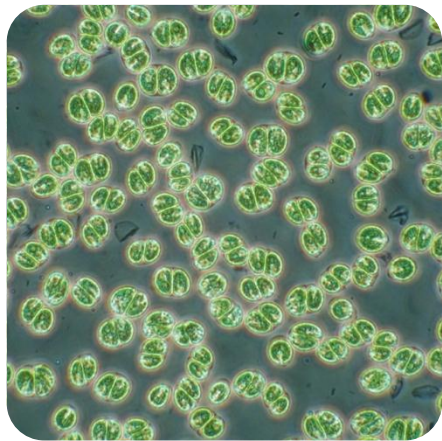


Production of specialties for food, aquaculture and non-food applications via multi-product biorefinery of microalgae

Overview and first results of the EU FP7 project MIRACLES

*Multi-product **I**ntegrated bio**R**efinery of **A**lgae: from **C**arbon dioxide and **L**ight Energy to high-value **S**pecialties (2013-2017)*

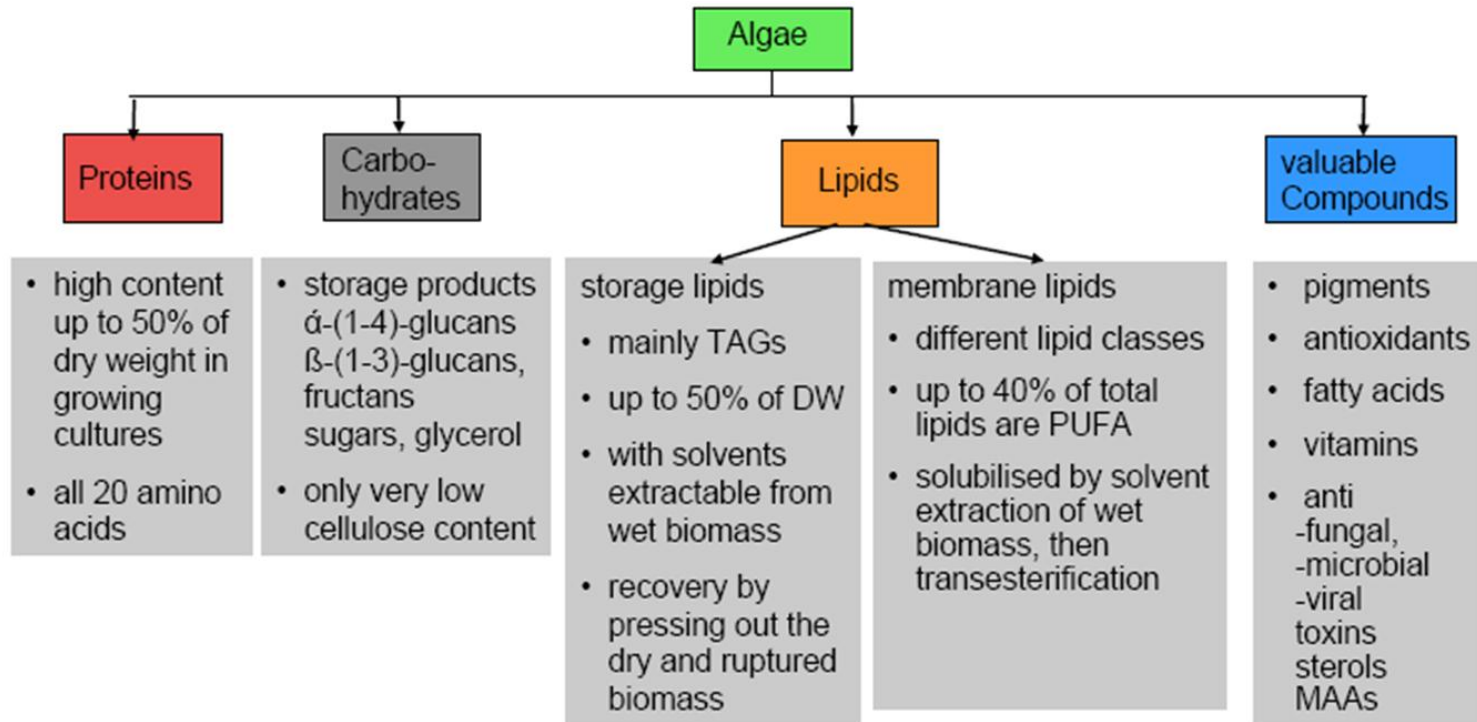
Hans Reith, Bert Lemmens, Hans Kleivdal, Lolke Sijtsma, Philippe Willems, Carlos Unamunzaga, Elke Breitmayer, Macarena Sanz, René Wijffels



1st EC Algae Contractors' Meeting, Florence, Italy, 2nd December 2014

Background

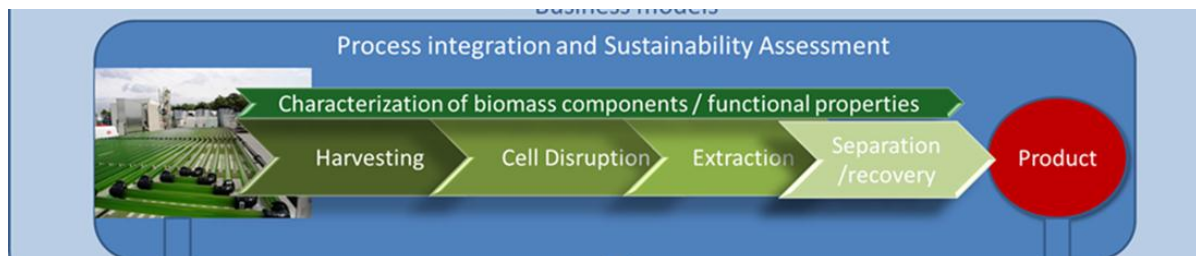
- Microalgae are a promising feedstock for sustainable supply of commodities and specialties for food and non-food products.



- Multiple products can be obtained via biorefinery, adding value to the biomass.

Aims of the project

- Limited implementation algal biorefinery to date is mainly due to unfavourable economics. Major bottlenecks:
 - high costs of algal biomass production
 - absence of appropriate biorefinery technologies
 - lack of concrete product applications
- MIRACLES aims to address these hurdles via:
 - enhancing cost-effectiveness of algae production and processing through technology development along the production chain
 - development of true multiple-product biorefinery technology for specialties from algae
 - development of new products for food, aquaculture and non-food



The MIRACLES CONSORTIUM: 26 partners

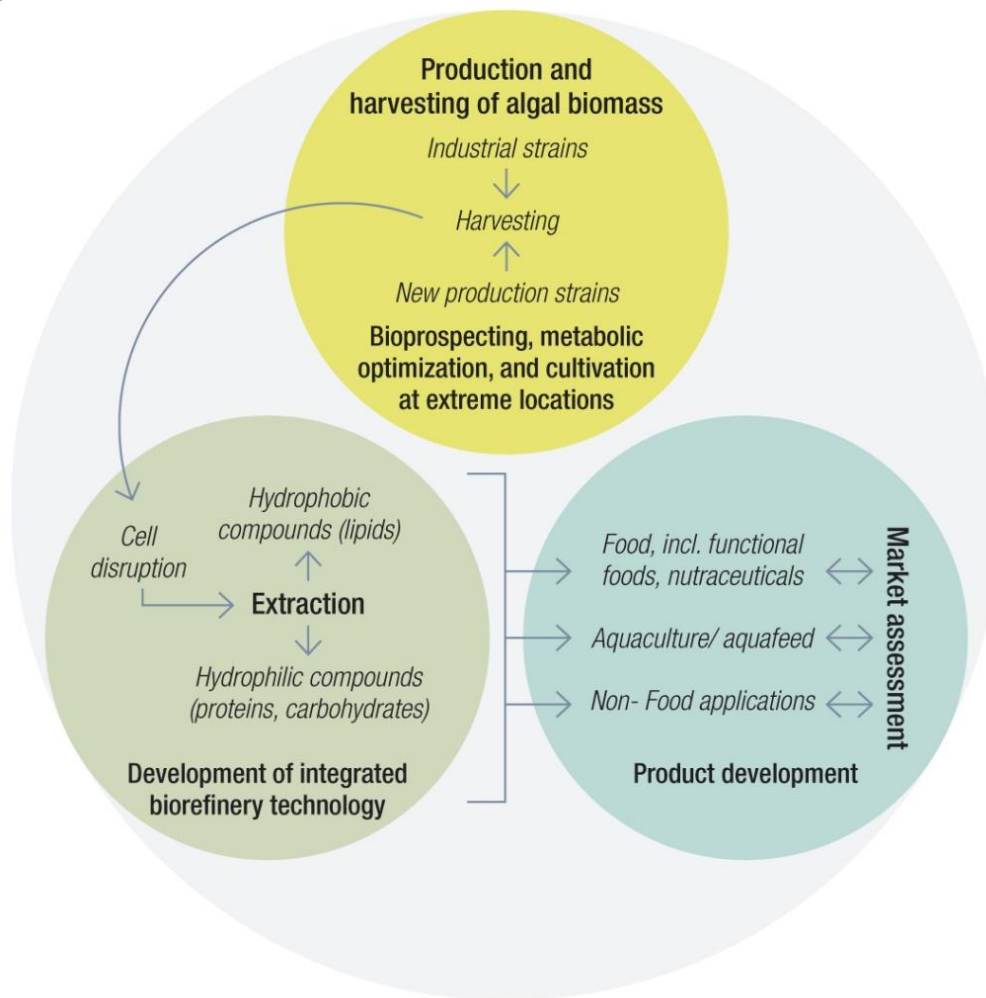
- Complementary expertise
- Strong industrial participation
- Commercial activities along the value chain



- 6 Universities, 5 Research Organisations,
- 12 SME's, 3 MNI's incl. 11 end user companies in target sectors
- In 6 EU countries + Norway + Chile.

Activities

←..... Demonstration of integrated value chains→
←..... Techno-economic and sustainability assessment→
integrated value chain & development of business plans



←..... Dissemination, exploitation & intellectual property management→
←..... Project coordination and overall management→

Production and harvesting (WP1)

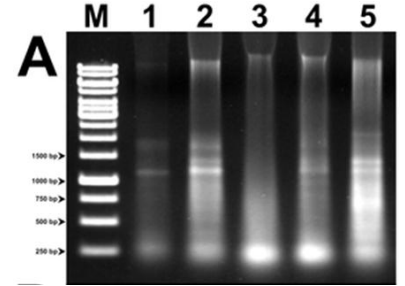
- **Production of biomass** for biorefinery, application RTD using established production strains by Fitoplancton Marino. *Nannochloropsis*, *Isochrysis*, *Phaeodactylum*, *Scenedesmus*.
- Strains can be removed or added during the project
 - Indoor photobioreactors (PBR) for pre-cultivation and modular outdoor production tubular Photobioreactors.
 - Total 36 outdoor production PBRs (2000L each) FITO is able to produce **up to 6 different strains in parallel**.
 - Pilot plant with 120 tubular PBR 400L each (top right).



Facilities Fitoplancton Marino
S.L. Cadiz, ES.

Development of molecular monitoring tools to optimize concentration of target biomolecules FITO,WU

- Aim: **optimise production and monitor the metabolic state of cells in real time**>> **enhanced production, quality control**
- First step in the biorefinery. Focus on *N. gaditana*.
- Ongoing: development of **molecular biology tools**



Next phase:

- Gene expression analysis
- Product optimization studies to establish correlation between culture conditions/gene expression/metabolite level: **molecular markers**

Kim B-H, Ramanan R, Cho D-H, Choi G-G, et al. (2012)

Innovative technology for CO₂ concentration from the atmosphere: UT (1) UNIVERSITEIT TWENTE.

- **Independence of CO₂ point sources** incl. flue gas. Enables cultivation in remote areas incl. deserts.
- **Low concentration inhibiting compounds** (NO_x, SO_x, particles)
- Cost target **<50-75 Euro/ton CO₂**
- **Identification of selective, stable sorbent.** Challenges: Low CO₂ concentration in air, water co-adsorption.

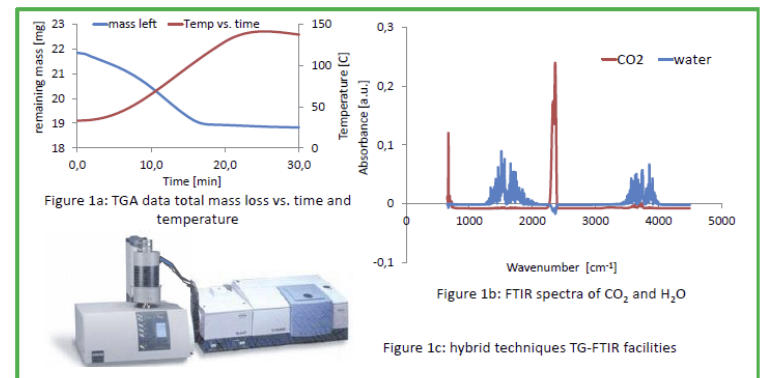


Figure 1: The peaks of CO₂ and water are clearly separated. The maximum peak for CO₂ is at $\nu=2360\text{ cm}^{-1}$ and for H₂O at $\nu=1510\text{ cm}^{-1}$. As the spectrum of CO₂ is easier to process, it is used for integration, the amount of H₂O adsorbed is subsequently determined by mass difference.

Innovative technology for CO₂ concentration from the atmosphere: UT (2) UNIVERSITEIT TWENTE.

- **Supported Amine Sorbents (SAS)** show higher capacity and selectivity for CO₂ adsorption compared to physi-sorbents and dry carbonate sorbents
- Sorbent characterization: saturation with water much faster than for CO₂ > H₂O does not inhibit active sites CO₂ sorption.

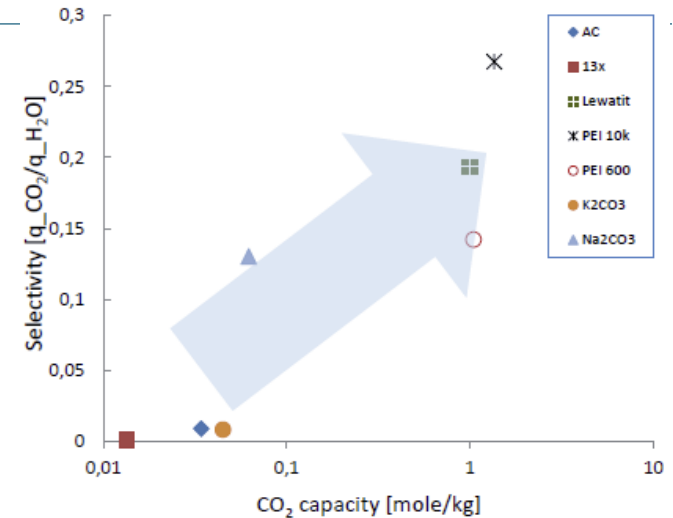


Figure 3a: screening sorbents for direct air capture at ambient (lab) conditions for 15h

- Next phase: **Development of selective desorption strategy**
- Final aim: **prototype and PoC on lab scale**

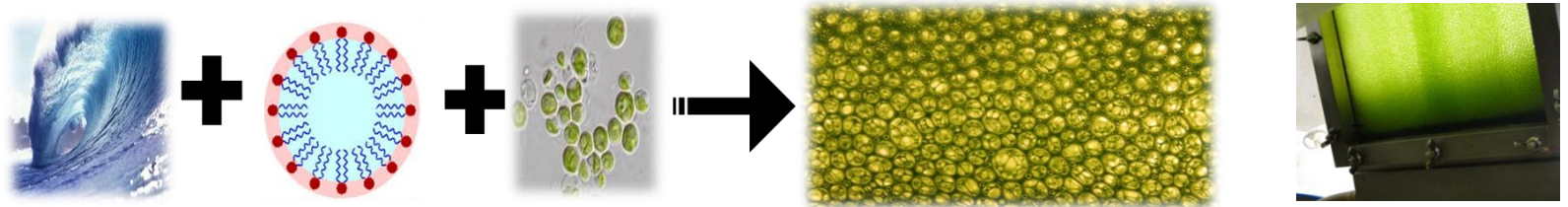
CO₂ capture from atmosphere for microalgae cultivation.
Qian Yu, Wim Brilman, 2014.
q.yu-2@utwente.nl

Development of novel Liquid Foam Bed flat panel PhotoBioReactor (WU, UHU)



Concept: growth of algae in liquid foams.

Foam generation controlled by foaming agents and gas distributors



Feature

Very short light path
Limited amount of water in reactor

Low weight, low pressure drop,

Large interface surface area
Increased gas residence time

Consequence

➤ High biomass conc. Reduced harvesting costs

➤ Reduced construction costs, energy costs

➤ Enhanced mass transfer CO₂, O₂

Overall: significant reduction of capital and operating costs



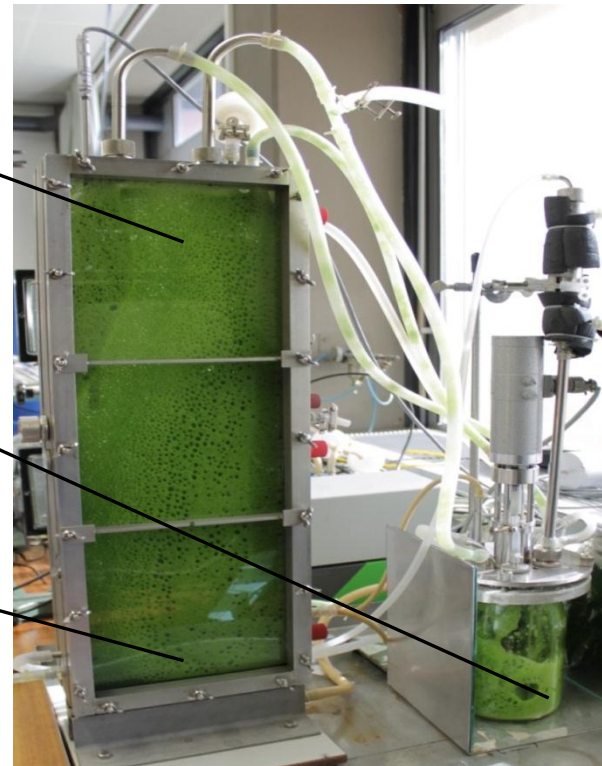
Liquid foam-bed photobioreactor (WU, UHU)

- A **working bench-scale liquid foam-bed photobioreactor was developed** using BSA as (experimental) surfactant.
- Growth demonstrated and good foam production and hold-up achieved with *C. sorokiniana* and *N. gaditana*.

Flat panel photobioreactor

Foam breaking device: gaseous phase is separated from the liquid phase.

Foam formation due to gas sparging



Liquid Foam Bed PhotoBioReactor

- Foam stability and liquid hold-up depend on surfactant concentration, gas flow rate, pH, T and salinity.
- **Ongoing:**
 - selection **alternative surfactants** with enhanced stability incl. toxicity testing
 - optimization **foam break up process**
- **Final aims:** indoor and outdoor optimisation, full-scale design



A. Janoska et al, 2014.

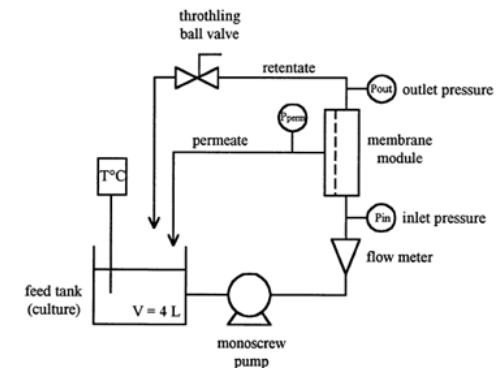
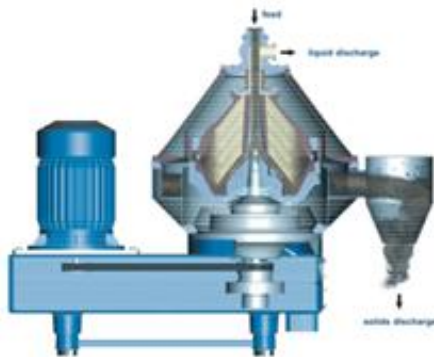
M. Vazquez et al, 2014. Production of stable microalgae-enriched foam.

Membrane based technology for harvesting and growth medium recycling VITO, TMUC (1)



THOMAS
MORE

- Harvesting and dewatering is a major cost and energy driver.

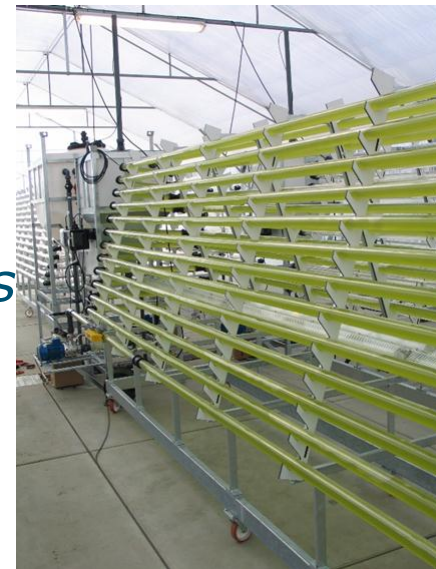


- Water and nutrients are a major cost factor in large scale cultivation.
- >>Medium recycling after harvesting is crucial for cost reduction
- Aim: **develop membrane technology and analytical methods capable of saving water, nutrients, energy and costs**

Membrane based technology for harvesting and growth medium recycling VITO, TMUC (2)

- Activities to date:
 - **Testing/comparison of membrane materials** for various algae
 - **Comparing configurations/ process conditions** with respect to suitability/performance for harvesting and medium recycle
 - Detailed **evaluation of nutrient requirements** and dosing in relation to recycling
- *More detailed info in presentation Bert Lemmens this afternoon 16:50 -17:50*

*Pilot scale facilities
TMUC, Geel, BE*



Bioprospecting, metabolic optimization, and cultivation at extreme locations (WP2)

- Partners: WU, FCPCT, UiB, UA, FITO, URDV, UniRes
- Objectives
 - to perform bioprospecting in extreme climatic conditions **to identify novel algal strains** with appropriate product profiles and biomass characteristics



- *Nordic and Arctic climate (Norway)*
- *Oceanic subtropical climate (extremophile environment) (Canary Isl.)*
- *Altiplanic lagoons and salt lakes (Chile)*

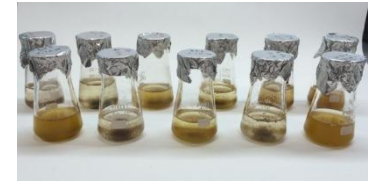
Extreme climate conditions push towards evolutionary adaptation
High biodiversity with special properties/components

- to optimize outdoor cultivation under different climatic conditions.
- to develop metabolic models to optimize productivity

Bioprospecting and screening



- First year **sampling programs performed**: high number of isolates obtained. Sampling ongoing.
- Development of **screening criteria** based on cultivation requirements (robustness,...) and input industrial partners
- **Screening of culture collections**: Bergen Marine Biobank, Spanish Bank Algae, ongoing



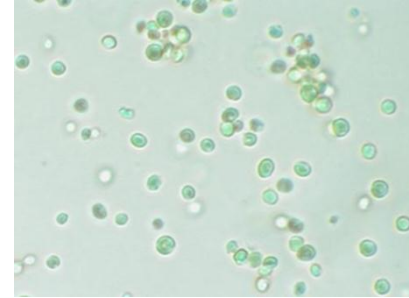
- Next phase: further sampling, screening, selection
- To evaluate and optimize indoor and outdoor production of selected strains under climatic extremes at partner locations

Metabolic modelling and optimization studies to maximize lipid production by *Nannochloropsis gaditana* (WU, FITO)

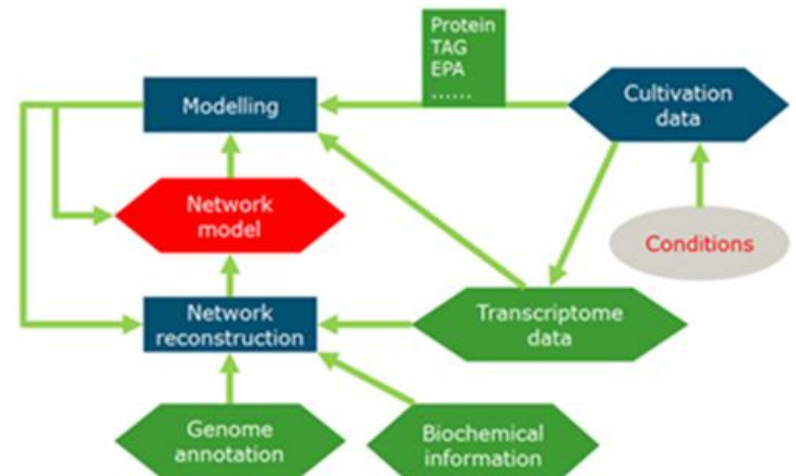
Ongoing: screening *N. gaditana* to study the **influence of different growth conditions** on productivity and yield of lipids

Next phase:

- **Comparing different growth conditions** using metabolic flux analysis and transcriptomics
 - Reveal regulatory mechanisms, bottlenecks for product formation
- >> Optimize productivity and yield
- >> Potential identification of targets for metabolic engineering

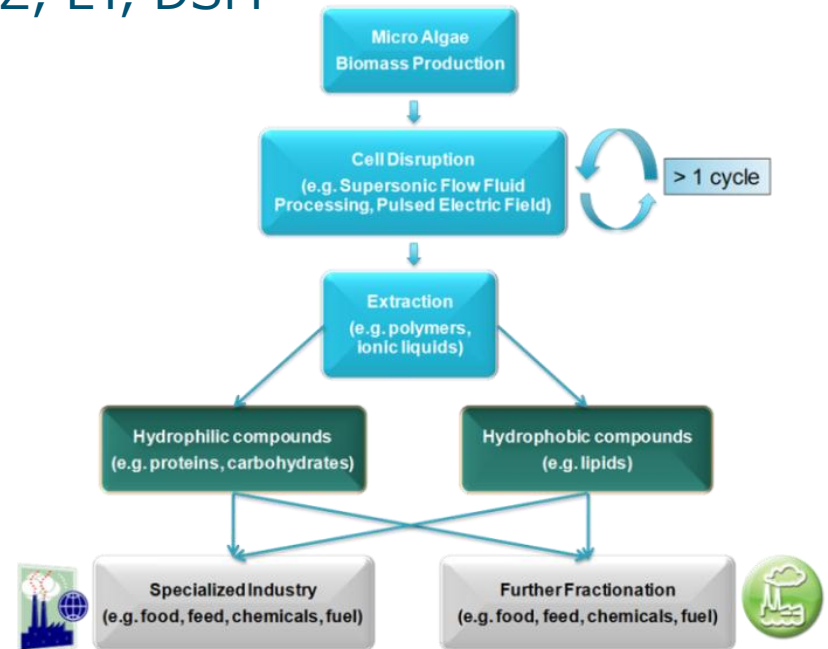


N. gaditana,
Genome sequence known.



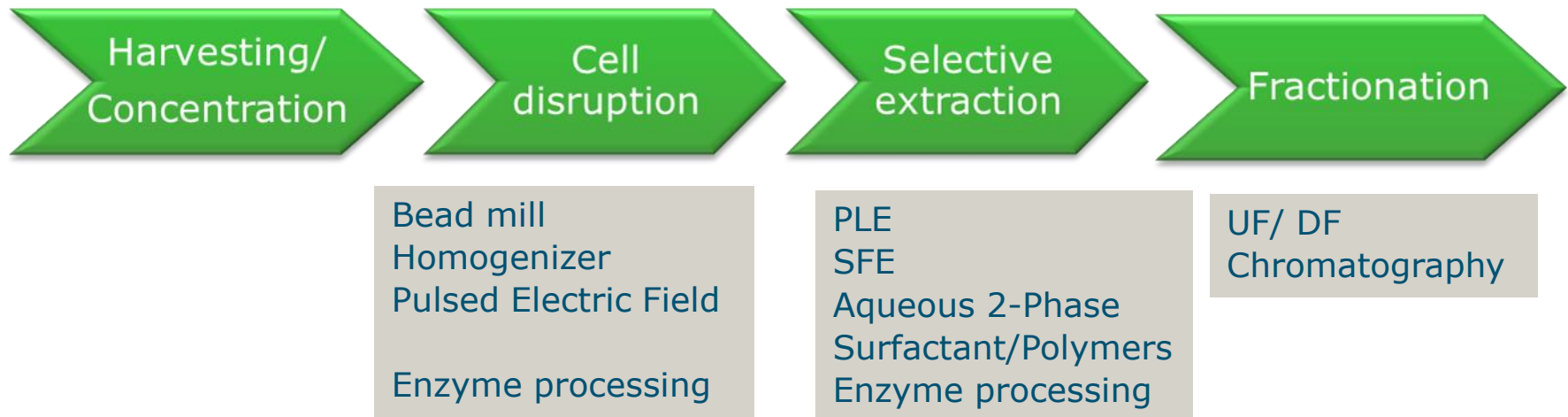
Development of integrated biorefinery (WP3)

- Development of integrated biorefinery / processing technologies employing **mild cell disruption, 'green' extraction and fractionation/ purification technologies** to produce **multiple specialty products** by valorising all biomass components
- Partners: WU, DLO, CSIC,DNL, IMENZ, ET, DSM



Development of integrated biorefinery (WP3)

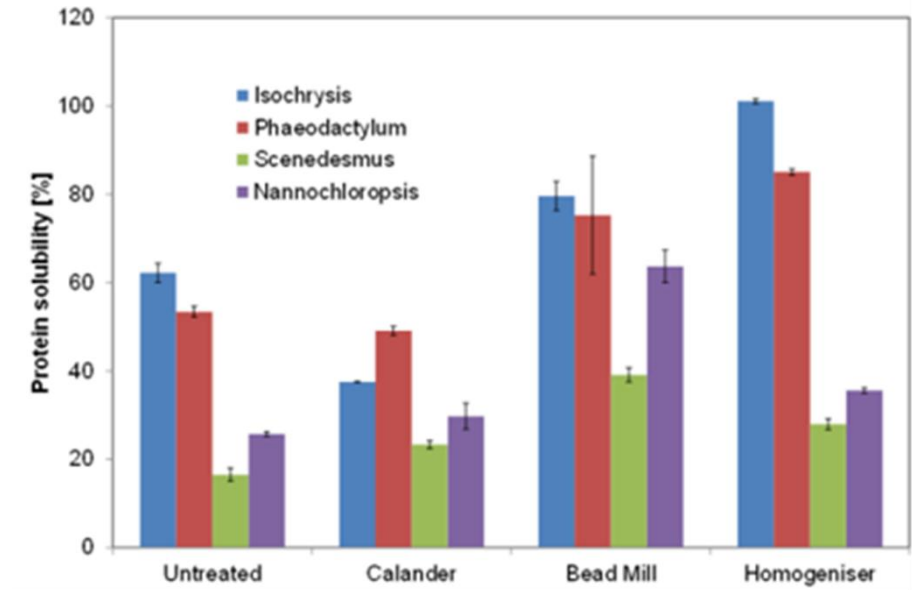
- need for **mild, yet effective technologies** preserving structure, functionality and value of **all fractions**
- technologies must be **environmentally friendly**, with low energy use



- processes must be **integrated into optimal biorefinery chains allowing continuous operation**

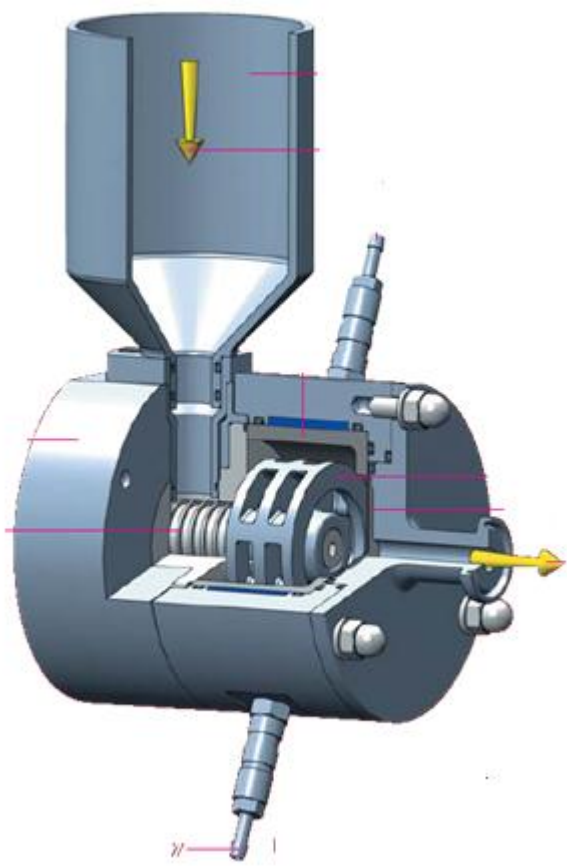
Integrated biorefinery: Preliminary results

- **Analytical methods established** and harmonised
- Biochemical **composition** of selected strains **established**
- Initial tests **cell weakening, cell disruption** performed

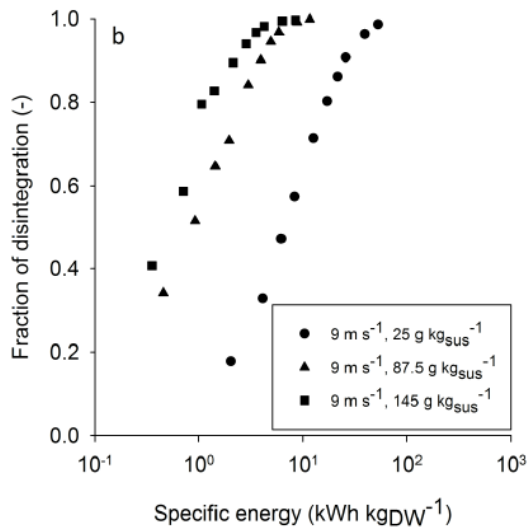
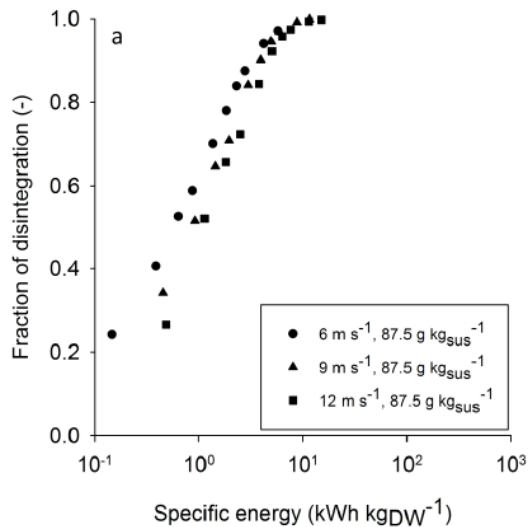
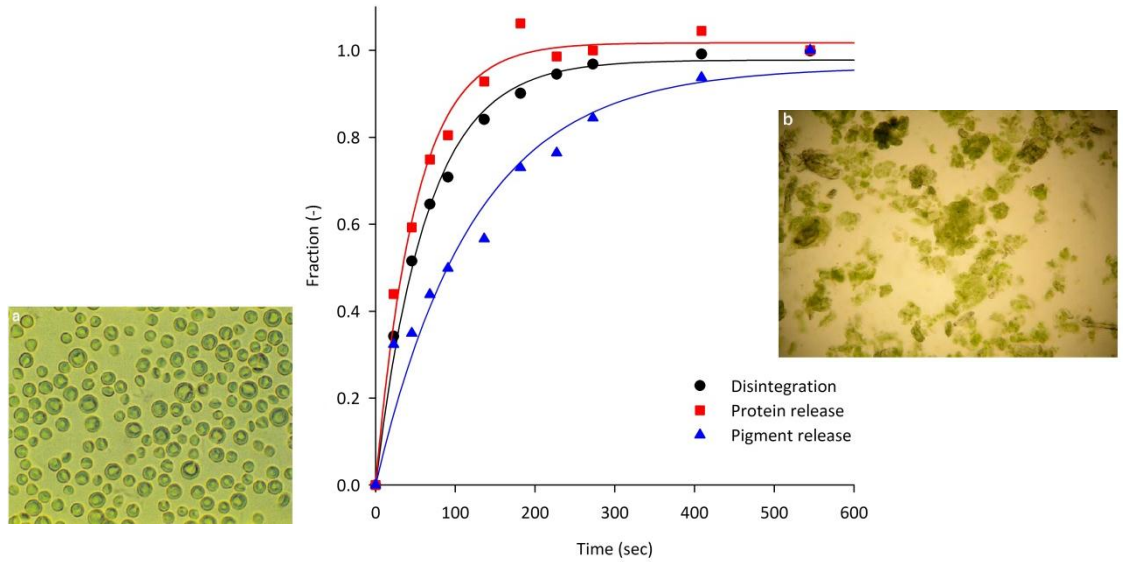


- Soluble protein is a useful marker for cell disruption monitoring
- Bead milling and homogenizer show best results to date
- Tests using enzymes show: significant degradation of cell walls, specific for different algae strains
- Preliminary **extraction technologies** have been tested

Cell disruption - Beadmilling

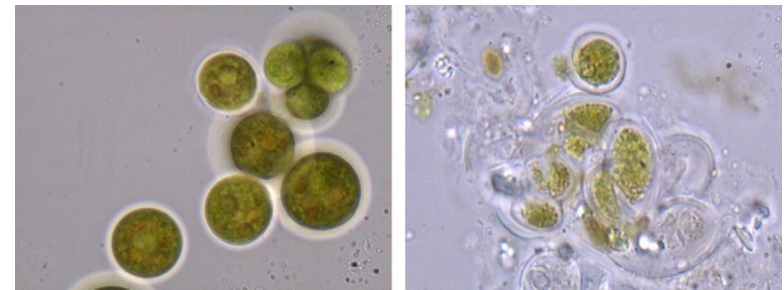
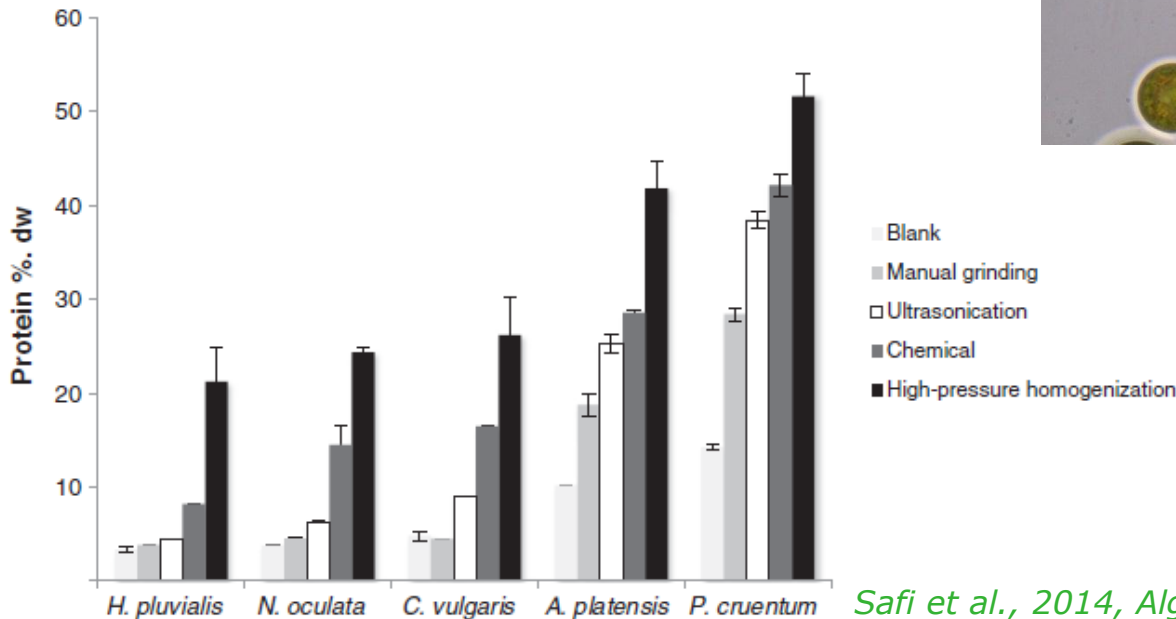
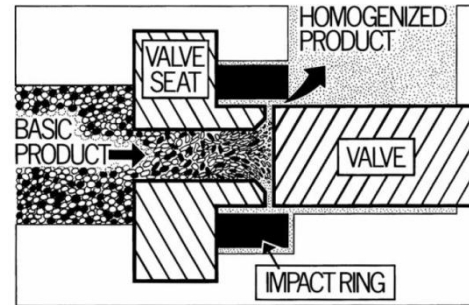


WAB Dynamill



Cell disruption - Homogeneizer

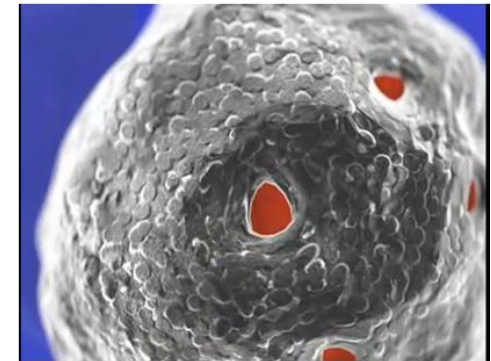
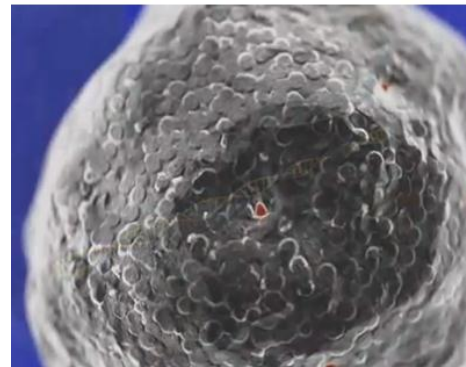
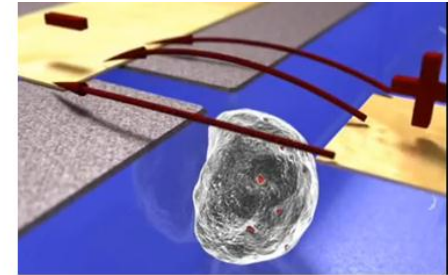
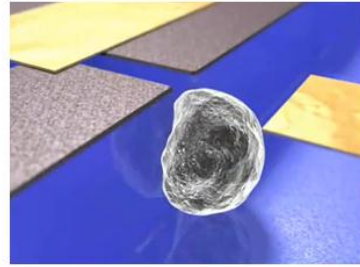
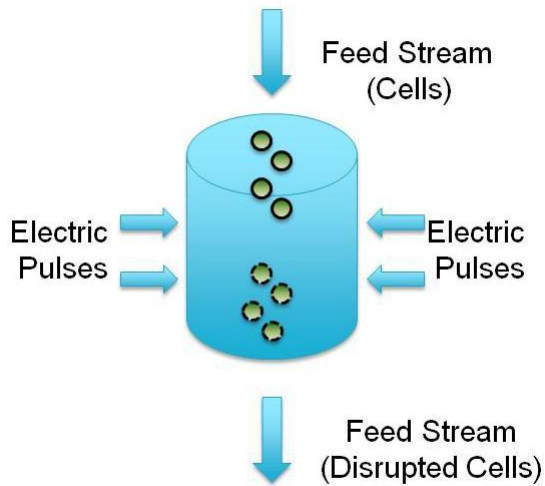
- 500-4000 bar
- Mechanism
 - Shear
 - Turbulence
 - Cavitation
 - Hit-Shock
- 100-1000 kWh/m³
- 2° C increase/ 100 bar/passage



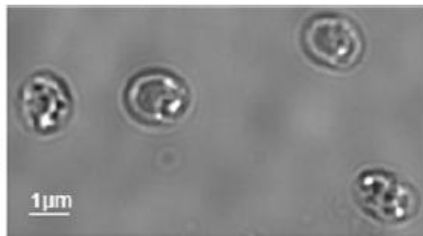
Halim et al., 2012, Appl Energy

Safi et al., 2014, Algal Res

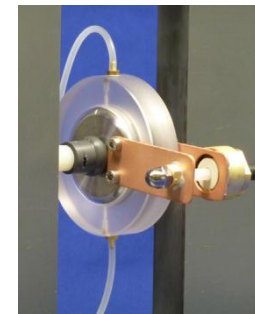
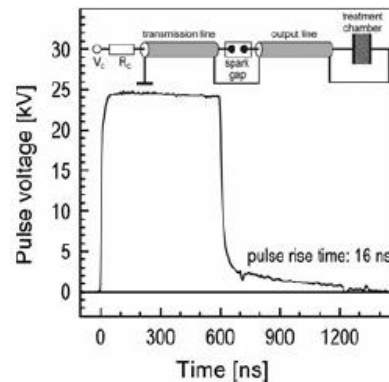
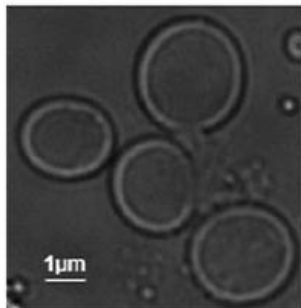
Cell disruption – Pulsed Electric Field



Before Electro extraction



After Electro extraction

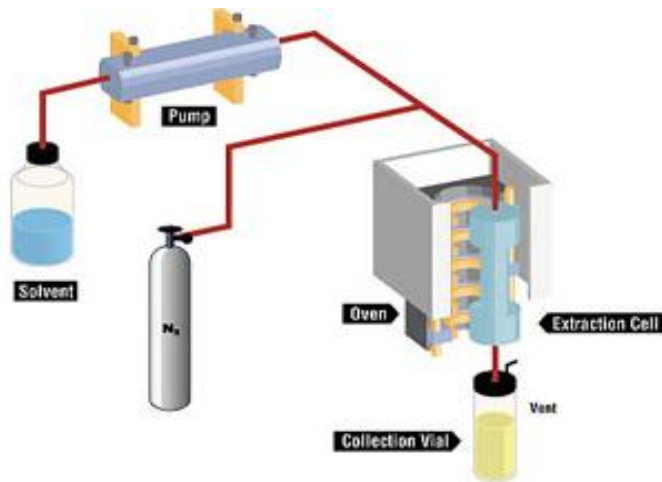


Cooustets et al., 2013, J Membr Biol

Goettel et al., 2013, Algal Res

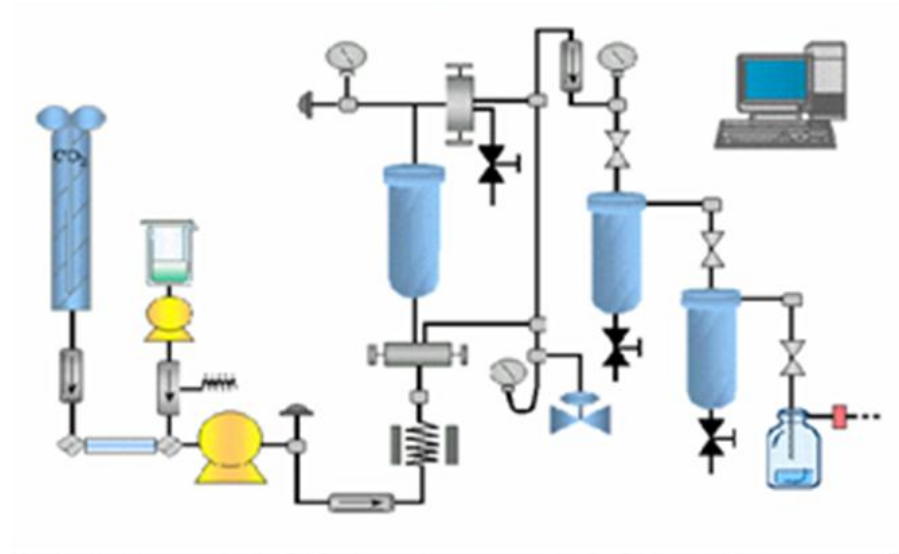
Pressurized Liquid Extraction (PLE) and Supercritical Fluid Extraction (SFE)

PLE



- Solvents maintained in liquid state (high temperatures and pressures)
- Faster extraction processes
- Low volumes used of solvents

SFE



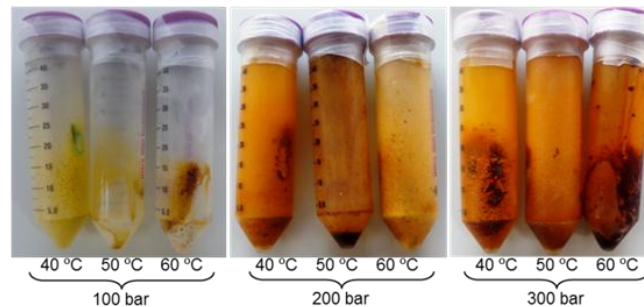
- CO₂ Extraction
- No oxidative damage
- Solvent-free product collected
- Products not in contact with toxic solvents

SFE first results



■ Sequential SFE extraction *Isochrysis galbana*:

- Neat CO₂ (extremely non-polar) to extract lipophilic compounds. Maximum yield and carotenoid content at 300 bar, 50C



- 2nd step using CO₂ with ethanol to obtain more polar compounds

M. Herrero et al, 2014. New green technologies to extract bioactives from Isochrysis galbana microalgae

Product development & market assessment (WP4)

- Partners: EWOS, SPAROS, BIOPOL, IMENZ, CHIMAR, VFT, NATAC, CE, URDV, DSM.
- **Aim:** To develop, validate and document the use of microalgae-derived products in
 - Food incl functional food, nutraceuticals
 - Aquaculture feeds
 - Selected non-food applications
- **Functionality testing, formulation and performance testing** of products in the lab and in pilot trials
- **Market assessment** to **validate** the proposed applications in the target sectors incl. product-market combinations, market data to position algae products vs existing reference products
- Interaction with **external stakeholders** on regulations, consumer acceptance,



Demonstration of integrated value chains (WP5)

- Aim: To demonstrate **integrated value chains** to **deliver proof-of-concept and demonstrate techno-economic viability**.
 - Pilot scale production of algae batches with optimized composition
 - **Validation** of selected processes and application testing at **pilot scale**
 - Demonstration of **4 best performing integrated value chains**
- Preparations ongoing
- Partners: WU, DLO, CSIC, EWOS, DNL, FITO, SPAROS, BIOPOL, CHIMAR, VFT, NATAC, ET, URDV, DSM

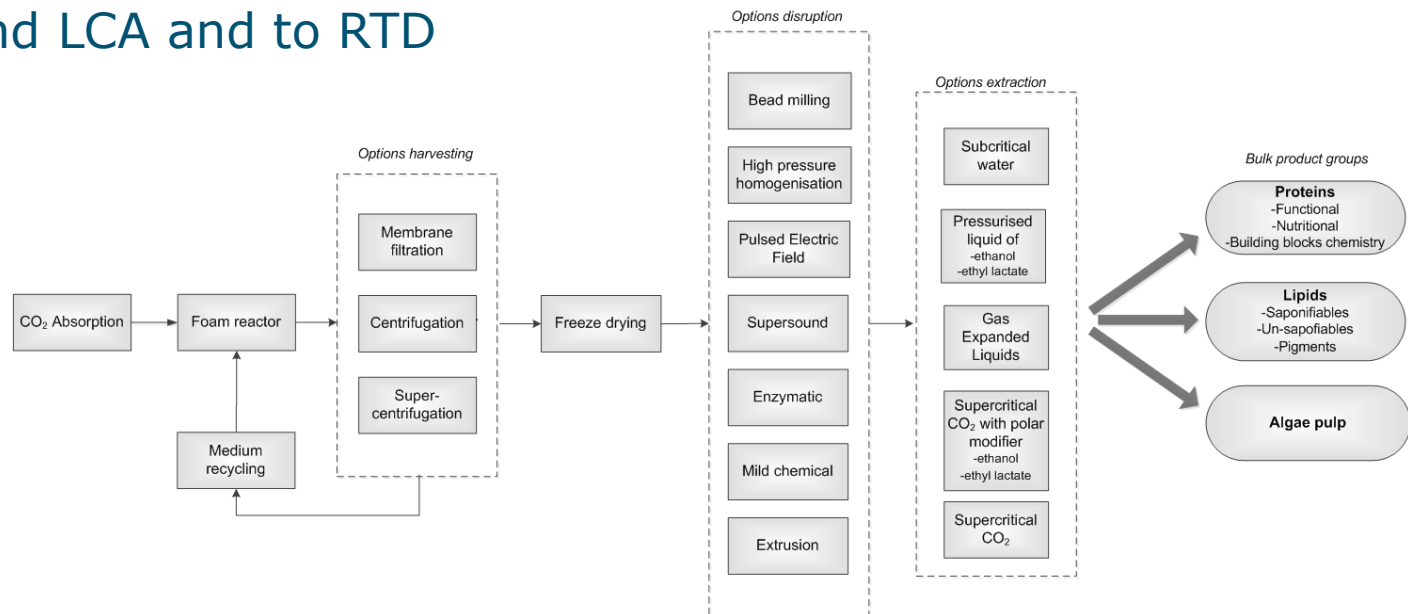
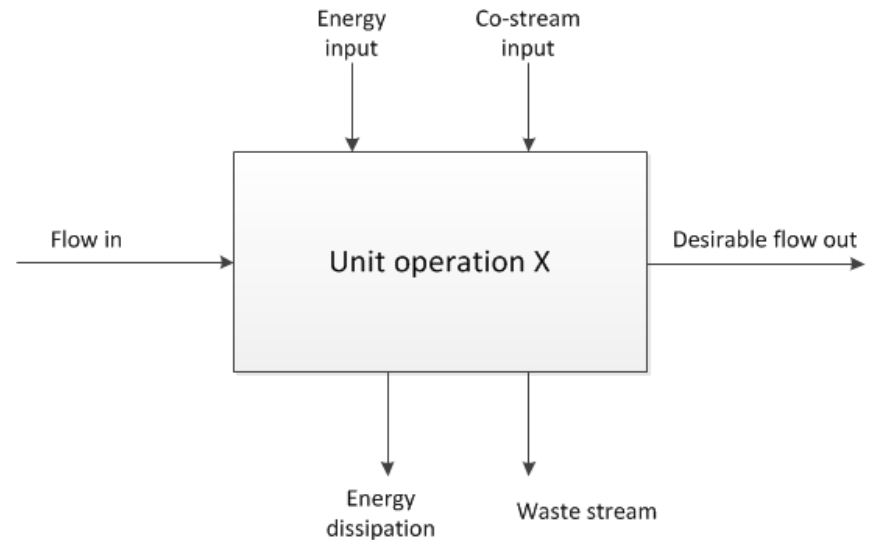
Techno-economic and sustainability assessment integrated value chain & development of business plans (WP6)

- To generate **conceptual biorefinery design models**
- To **assess the economics and sustainability** of the biorefinery concepts employing **techno-economic evaluation, Life Cycle Assessment (ISO)** and **socio-economic assessment**
- **Economic evaluation of scenarios for multi-product biorefinery** value chains for high-value specialties and scenarios for co-production of specialties and algal biofuels
- **Fully documented business plans**

- Partners: WU, VFT, nova, ET with input by all partners

Approach

- Mass and energy balance models
- Evaluation of the process chain with mutual interactions
- Specifying yields, resource and energy requirements, emissions
- Link / feedback between process simulation and LCA and to RTD



Acknowledgements



- The project is supported by the European Commission through the 7th Framework Program under Grant Agreement No. 613588.
- Program Officer DG R&I: Garbiñe Guiu, garbine.guiu@ec.europa.eu
- For more information: Hans Reith, hans.reith@wur.nl
- Project website: www.miraclesproject.eu