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


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

Production and harvesting of algal biomass
Industiral strains
Harvesting
New production strains
Bioprospecting, metabolic optimization, and cultivation at extreme locations

Cell disruption
Hydropobic compounds (lipids)
Extraction
Hydrophlic compounds (proteins, carbohydrates)
Development of integrated biorefinery technology

Food, incl. functional foods, nutraceuticals
Aquaculture/aquafeed
Non-food applications
Product development

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).
**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**

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

- **Independence of CO₂ point sources** incl. flue gas. Enables cultivation in remote areas incl. deserts.
- **Low concentration inhibiting compounds** (NOₓ, SOₓ, 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 ν=2360 cm⁻¹ and for H₂O at ν=1510 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 CO\textsubscript{2} concentration from the atmosphere: UT (2)

- **Supported Amine Sorbents** (SAS) show higher capacity and selectivity for CO\textsubscript{2} adsorption compared to physi-sorbents and dry carbonate sorbents.
- Sorbent characterization: saturation with water much faster than for CO\textsubscript{2} > H\textsubscript{2}O does not inhibit active sites CO\textsubscript{2} sorption.
- Next phase: **Development of selective desorption strategy**
- Final aim: **prototype and PoC on lab scale**

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**CO\textsubscript{2} capture from atmosphere for microalgae cultivation.**

Qian Yu, Wim Brilman, 2014.

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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.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Consequence</th>
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<tr>
<td>Very short light path</td>
<td>High biomass conc. Reduced harvesting costs</td>
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<tr>
<td>Limited amount of water in reactor</td>
<td></td>
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<tr>
<td>Low weight, low pressure drop,</td>
<td>Reduced construction costs, energy costs</td>
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<tr>
<td>Large interface surface area</td>
<td></td>
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<tr>
<td>Increased gas residence time</td>
<td>Enhanced mass transfer CO2, O2</td>
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Overall: significant reduction of capital and operating costs.
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

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

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

Extreme climate conditions push towards evolutionary adaptation

**High biodiversity** with special properties/components

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

![Diagram showing the process flow of biorefinery](chart.png)

- **Harvesting/Concentration**
  - Bead mill
  - Homogenizer
  - Pulsed Electric Field
  - Enzyme processing

- **Cell disruption**

- **Selective extraction**
  - PLE
  - SFE
  - Aqueous 2-Phase
  - Surfactant/Polymers
  - Enzyme processing

- **Fractionation**
  - UF/DF Chromatography

• 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 - Bead milling

WAB Dynomill

Postma et al., 2014, Bioresour Technol
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 Energ

Safi et al., 2014, Algal Res
Cell disruption – Pulsed Electric Field

Coustets et al., 2013, J Membr Biol
Goettel et al., 2013, Algal Res
Pressurized Liquid Extraction (PLE) and Supercritical Fluid Extraction (SFE)

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

- 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, 50°C
  - 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*
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
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