MIRACLES SPECIALTIES FROM ALGAE

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Newsletter

Editorial Hans Reith, Project Coordinator

Successful scale-up of microalgae biotechnology requires reduction of production costs and enhancement of the economic output. The overarching objective of the MIRACLES project (2013-2017) was to contribute to this goal via innovation and technology development in algae production and processing and the development of new products. The project results are now available and they show that MIRACLES has successfully developed and demonstrated:

- technological innovations that contribute to improving cost-effectiveness of algae production, harvesting and processing;
- multiproduct biorefinery concepts and processes with a profitable business potential;
- a range of new specialty products for application in food, aquaculture and non-food.

The project has achieved practically all objectives and generated numerous exploitable results, ready for further development and commercialization. This Newsletter presents the scientific and technological progress in the final year of the project and the highlights in the area of product development. Please contact the consortium via one of the contact persons indicated below for more information.

In the coming period the project results will be made available in more detail via publications and in the form of a public summary report by the European Commission and via the project website.

Project website

http://miraclesproject.eu/

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Progress and Major Results in the Final Project Period

Production and harvesting of algal biomass (WP1)

In WP1 partners' activities focused on improvement of production and harvesting of algal biomass to achieve substantial cost reduction through: (1) Development of tools and strategies to optimize the concentration of target biomolecules in the algal biomass; (2) A new technology for CO_2 concentration from the atmosphere; (3) A novel photobioreactor concept and (4) Innovative membrane based technology for combined harvesting and medium recycling *(Figure 1).*

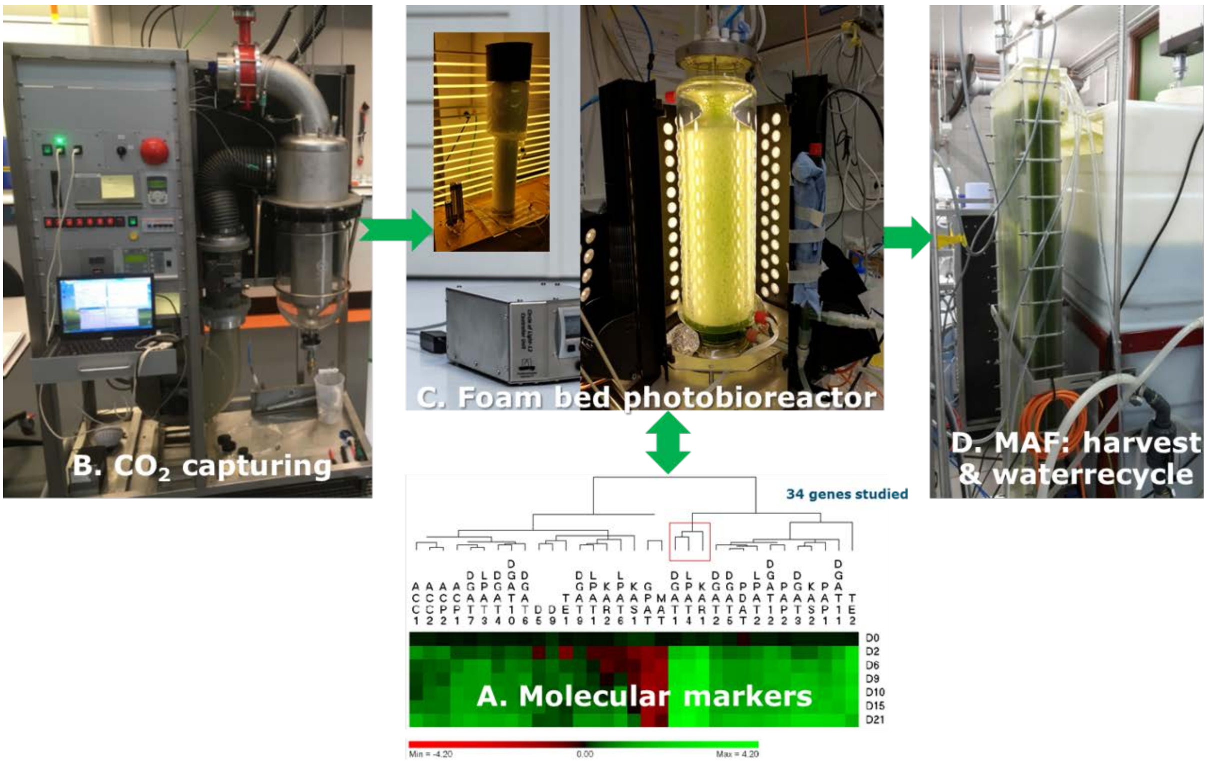

Figure 1. Technology & tools successfully developed for improvement of algae cultivation & harvesting

In the final project period, Fitoplancton delivered freeze-dried, frozen as well as high-pressure disrupted algae biomass to partners for processing and product application R&D. Furthermore Fitoplancton performed pilot trials with *Nannochloropsis gaditana* under nitrogen starvation in outdoor photobioreactors to induce oil/TAG (Tri Acyl Glycerides) accumulation in the algae biomass. The oil was extracted by partner EcoTreasures and further developed into products (aqua feed). The trials also revealed that **molecular markers** LPAT4 and DGAT1 are appropriate indicators to monitor TAG enrichment in the algae biomass. A

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similar molecular tool was developed for monitoring and control of the omega-3 fatty acid EPA (Eicosa Pentaenoic Acid) in *N. gaditana* biomass. These tools can be used in industrial microalgae production for:

- Real-time monitoring of TAG and EPA content for appropriate timing of biomass harvesting to reach optimal productivity.
- Selection programs for clones with higher capacity and/or efficiency to accumulate TAG and EPA.

Development of technology for CO_2 concentration from the atmosphere (WP1.2 – UT) aimed at realizing supported amine based technology for CO_2 capture from air at a suitable concentration for algae cultivation at minimal costs. The focus in the final period was on (i) the performance evaluation of the designed and built **Radial Flow Reactor (RFR) for CO2 adsorption from air**; (ii) the design/construction and testing of a suitable regeneration strategy; (iii) sorbent degradation studies and (iv) process evaluation. Process optimization and –evaluation studies were performed. A lab-scale operating unit providing a Proof of Concept for concentrating CO_2 to a 1% CO_2 in air stream and producing 0.5 kg of CO_2 per day was designed, constructed and demonstrated in combination with algae cultivation in October 2017.Process evaluation showed that the operating costs for CO_2 production from ambient air are ca.75 €/ton CO_2 when producing a CO_2-enriched air stream and ca. 120 €/ton CO_2 for pure CO_2.

The work on development of a novel Liquid Foam Bed Photobioreactor concept (WP1.3 – WU& UHU) aimed for significant cost reduction due to lower reactor weight, improved gas exchange and high biomass density. Partner WU successfully tested an optimized 'generation 3' foam-bed photobioreactor at laboratory scale which enabled to apply Pluronic F68 as a surfactant for at least 20 days. The recirculation rates of the algal suspension remained low, and according to model calculations the energy requirement for a hypothetical large-scale foam bed photobioreactor is reduced by an order of magnitude in comparison to a comparable suspension based photobioreactors. High biomass concentrations up to 23 $g \cdot L^{-1}$ were reached. Model calculations showed that carbon dioxide uptake efficiency can be maximized to more than 95%. The effect of Pluronic F68 on the biochemical composition of microalgae was investigated by UHU further in both nutrient replete and nitrogen-starved liquid cultures, using 4 newly built units of the lab-scale foam-bed photobioreactor prototype (previously designed at UHU). Batch cultivation of *Chlorella* in foam was found to lead to increased carbohydrate to lipid ratios.

Development of innovative harvesting and medium recycle technologies (WP1.4 – VITO, TMUC) aimed to reduce costs and energy use through the development of membrane technology for combined algae harvesting and water and nutrients recycling. In the final project period, **medium recirculation during pre-harvesting** (before the centrifugation step) was further studied and applied, using the submerged Membrane-based Algae Filtration (MAF) technology of VITO. On the one hand, VITO improved and evaluated the MAF-device, while TMUC performed growth experiments with *N. gaditana* in the recycled medium. The latter experiments showed that, with regular supplement of nutrients, the microalgae culture can be maintained in continuous harvest mode for several weeks. VITO & TMUC performed a long–term continuous growth & harvesting test at pilot scale (1500L reactor) with an average observed medium recycle of 92%. It was calculated that use of the MAF-system reduces significantly the OPEX (energy, water, salt, reduced discharge of water) as well as the CAPEX of the algae growth & harvesting process.

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Bioprospecting and selection of robust, highly productive algal species from extreme and diverse climatological conditions (WP 2)

The aim of this work is to enable cultivation of algae in areas with limited potential for agriculture, and broaden the resource base of the microalgae industry by finding new production strains with potential application in various market segments.

The first task focused on bioprospecting and strain selection from microalgae under climatological extremes in Norway, Spain, and Chile. Aim was to find robust, highly productive algal strains with appropriate biomass characteristics at extreme climatological conditions through a fit-for-purpose bioprospecting and strain selection program. In previous reporting periods environmental samples were gathered at each location. From theses environmental samples, clonal cultures of the most promising isolates with high growth potential were developed. This process was continued during the final period, and further isolates were screened for high lipid, PUFA content and composition, β-glucans, polysaccharide, antioxidants and proteins. Results are shown in *Table 1*.Promising isolates were further characterized to evaluate the commercial potential within the various application areas.

Table 1 Bioprospecting and the resulting samples in subsequent steps in the three locations: Gran Canarias, Spain, Antofagasta, Chile and Bergen, Norway.

	Environmental samples collected	Enrichments cultures in lab	Clonal isolates established for cultivation	Screened strains	Candidate strains
Subtropics **Gran Canarias, Spain**	213	639	154	24	9
Desert **Antofagasta, Chile**	69	360	66	20	15
Arctic/Nordic **Bergen, Norway**	58	110	149	29	8
Total	**340**	**1109**	**369**	**73**	**32**

The biomass production of selected algal strains was evaluated in pilot-scale outdoor photobioreactors (Green Wall Panel; GWP) under different climatological conditions at partner locations (Figure 2).

From the eight selected promising species from Norway, the first two species had already been successfully tested, and in the final period another three species were scaled-up to outdoor production experiments and compared with the earlier results. The three locally isolated strains of *Phaeodactylum Tricornutum* (B58, M28 and M29) and one *Entomoneis* sp. (M122) grew well under outdoor conditions. Both B58 and M29 are considered to be potentially interesting strains with high biomass productivity and an interesting biomass composition (especially EPA content).

From the outdoor cultivation experiments in Gran Canaria it is clear that all selected strains from that location demonstrate a potential high content of the different metabolites studied. Most interesting were the *Euglena* strains. *Euglenas* are a good candidate to produce at large scale because of the high amount of insoluble ß-glucans, polysaccharides with a high medicinal and economic potential.

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From the strains bioprospected in the Atacama desert and along the coast of Antofagasta in Chile that were deemed potentially interesting, four were able to grow successfully in outdoor photobioreactors: *M. inermum* strain 42.2 (proteins); *M. inermun* strain 44.2 (carbohydrates); *Chlorella* sp. strain 47.3 (proteins); and *Nitzschia draveillensis* strain 53.3 (fucoxanthin). For these strains, the productivity was optimized after three growing cycles in the GWP. The *N. draveillensis* strain 53.3 diatom showed the highest growth in the GWP. Combined with its high content of fucoxanthin and EPA, which complements its antioxidant activity, this strain has high potential for the food industry.

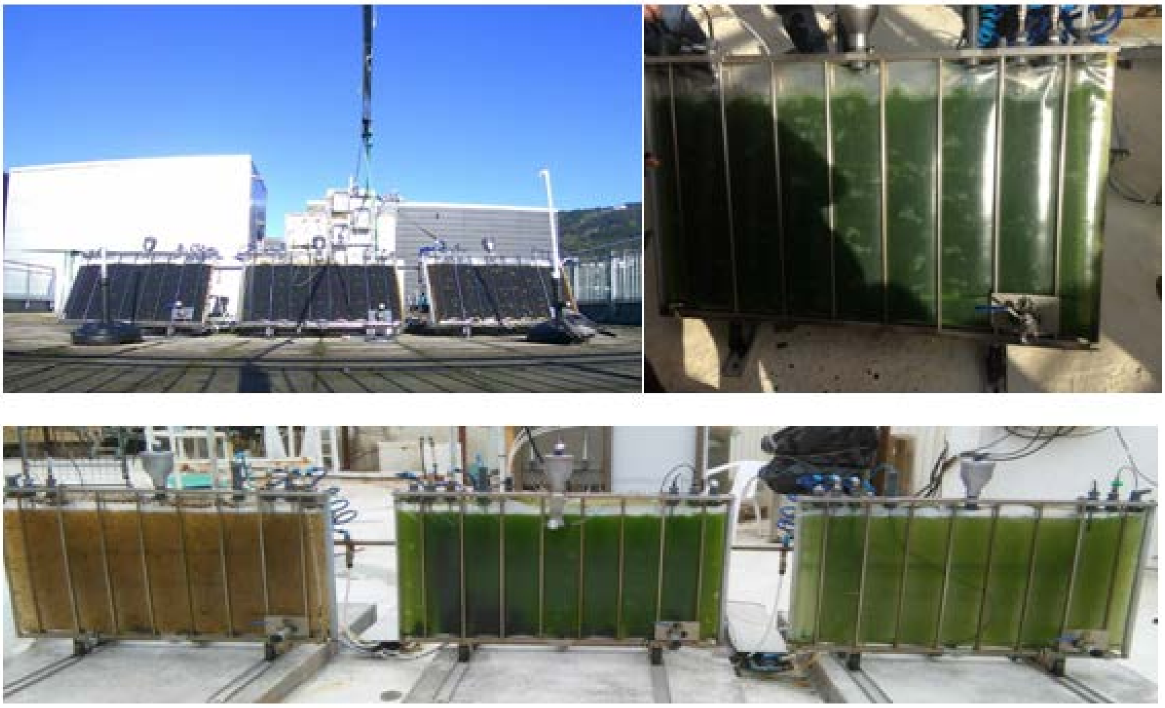

Figure 2. Pilot-scale photobioreactor set-ups at Bergen, Norway (upper left), Mejillones, Chile (upper right), and Gran Canaria, Spain (bottom).

In the area of metabolic modelling and studies to maximize product yields, WU has studied the mechanisms of EPA production by using 13 C labelling and transcriptomic analysis of well controlled cultures in the lab under nitrogen starvation but also of outdoor cultivation. New insights in fatty acid accumulation in TAG and polar membrane lipids during nitrogen starvation were obtained, especially for EPA.

Development of integrated biorefinery technology (WP 3)

The aim of WP3 was to develop integrated biorefinery technologies employing mild disruption, green extraction and fractionation/purification technologies to produce multiple products from algae biomass by valorising all biomass components (Figure 3)

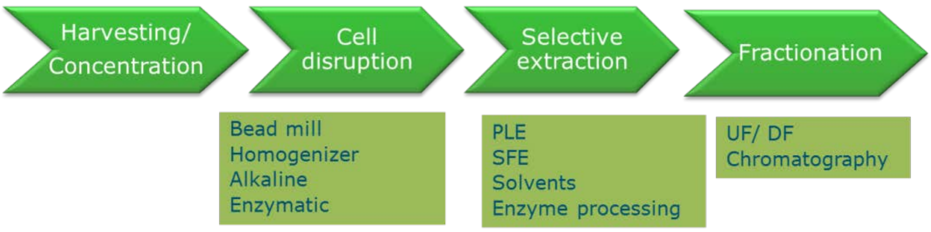

Figure 3. Process steps in the algae biorefinery

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For extraction of valuable compounds it is important to know the composition of the biomass which may vary depending on species, strain and cultivation conditions. The composition of the four algal strains used in MIRACLES: *Nannochloropsis gaditana, Phaeodactylum tricornutum, Isochrysis galbana* and *Scenedesmus obliquus* was determined. In addition, the chemical composition of algal strains from different climatic environments isolated by consortium partners was determined. Protein was the main fraction for all species investigated followed by lipid/oil, ash and a sugar fraction (*Figure 4*).

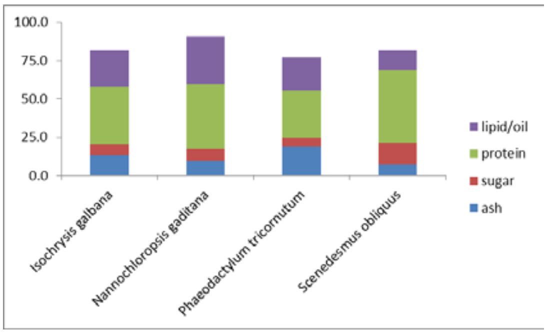

Figure 4 Ash, sugar, protein and lipid/oil content of the microalgae based on dry weight % (w/w).

Cell disruption is needed to release valuable products from the microalgae. An overall comparison between different cell disruption methods was made for *N.gaditana*. Cell disintegration, protein yield and the specific energy input were taken into account. High-pressure homogenization (HPH) and bead milling were the most efficient with low energy input and released ±50% protein (w/w). (*Figure 5*) Enzymatic treatment (protease) required low energy input but it only released ±35% protein (w/w). Pulsed Electric Field was neither energy-efficient nor successful for protein release and cell disintegration for the strain tested. Incubation of algal biomass at elevated temperatures and high pH (pH 12) was efficient in cell disruption. Such a pre-treatment, however, is limited to applications that do not require protein functionality. Tests were conducted to identify enzymes from bacteria, (marine) fungi and microalgae able to improve the cell disruption of microalgae.

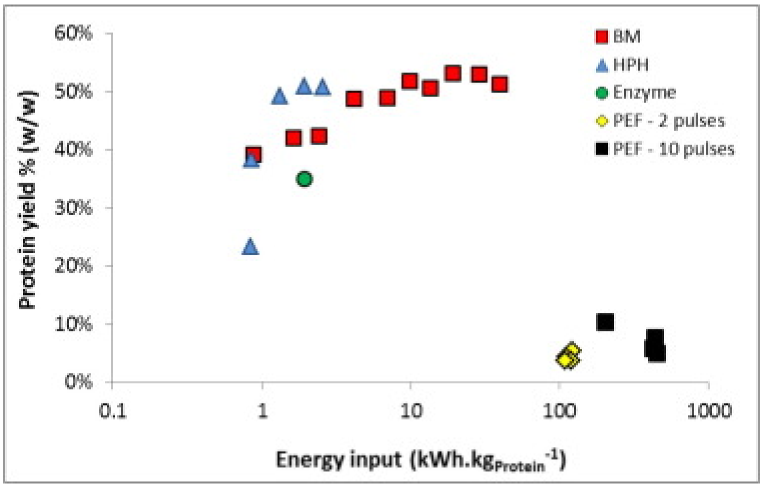

Figure 5. Overall comparison between the cell disruption methods tested with Nannochloropsis gaditana. The results displayed represent the protein yield and the specific energy input (E). BM is bead milling, HPH is high pressure homogenization, PEF is pulsed electric field. (From Safi et al, 2017)

Extraction and purification of lipids, proteins and bio-actives were investigated in more detail. Lipid extraction was performed with $ScCO_2$. Two sequential extraction steps were studied: 1) extraction with neat

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CO_2 (extremely non-polar) to extract highly lipophilic compounds and 2) extraction with CO_2 and ethanol as co-solvent. Cell disruption was important for oil extraction. For practical reasons, to produce sufficient material for application research, lipids were extracted on pilot scale with two different solvent systems: (i) hexane/isopropanol (ratio 3/2) and (ii) hexane/ethanol (ratio 92/8). Extraction efficiency of > 80% of the oil was obtained at pilot scale.

A mild biorefinery process was performed for the microalgae *N.gaditana*. After optimizing the process conditions, the combination of protease followed by ultrafiltration /diafiltration resulted in a higher overall yield of water soluble proteins in the permeate compared to the combination of HPH followed by ultrafiltration/diafiltration.

A downstream processing platform has been developed to extract bioactive compounds from the microalgae *I.galbana* using various pressurized green solvents. Extractions were performed in four sequential steps using (1) $ScCO_2$, (2) $ScCO_2$/ethanol, (3) pure ethanol, and (4) pure water as solvents, respectively. The results obtained showed that the extraction process was partially selective according to the polarity of the solvent/mixture of the solvents used. Furthermore, extraction and fractionation of bioactive compounds from the microalgae *S. obliquus* were performed. The process involved the following steps: (1) supercritical fluid extraction (SFE) ScCO2; (2) gas expanded liquids (GXL) using 75% ethanol and 25% ScCO2 (v/v) and; (3) pressurized liquid extraction (PLE) using water. Triacylglycerols were mainly extracted by SFE. Lutein and β-carotene were the main pigments which were preferentially extracted in the GXL step. Polar compounds such as proteins and sugars remained predominantly in the residue. Therefore, the green downstream platform developed in MIRACLES for valorisation of the microalgae biomass, is able to produce different fractions with potential application in the food, pharmaceutical and cosmetic industries

Five single product chains and 3 multiple product value chains were modelled in SuperPro Designer®. A specific model for each biorefinery chain was designed at industrial scale on basis of a combination of novel technologies/unit operations developed in MIRACLES in WP3 together with benchmark technologies when required from the product specification. The results obtained at lab and pilot scale, were further scaled up to a representative production scale of 10,000 tons y^{-1} of microalgal biomass. Based on the models, it can be concluded that the yield of biomass exploitation to marketable product has increased from 7-30% for a single product value chains to >95% for multiproduct value chains.

Product Development and Market Assessment (WP4)

In WP4: Product Development and Market Assessment, the incorporation of microalgae as whole cells or as extracts was studied in different applications. The highlights are presented here.

Food applications

The main result in Food application was obtained by URDV with crude protein fractions having good emulsifying properties. Several emulsions were stable for at least several weeks with a spoonable, mayonnaise-like consistency, a pleasant smell and attractive green colour. To come to a marketable solution, more research is needed on the production process and characterisation of those proteins.

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Aquafeed applications

Partner SPAROS has focused its research efforts towards identification of benefits above and beyond the basic nutritional needs of fish. Feeding trials were conducted with gilthead sea bream and Senegalese sole. Major results were:

- Diets containing 5% of broken-cells extract of the fucoxanthin-rich microalgae *P. tricornutum* significantly enhanced the survival of sole larvae and reduced the skeletal malformation rate.
- A diet containing 1% of *P. tricornutum* improved non-specific immune response to stressful events in sole and sea bream juveniles.
- Adult sea breams fed with the microalgae-rich diet had a lower fat accumulation, a better external appearance and higher brightness. A consumer panel characterized algae-fed seabream as having a more vivid and typical external pigmentation

Partner EWOS/Cargill performed digestibility and salmon growth trials to indicate nutritional value of phototrophic algae. Overall growth performance was not inferior to a reference diet, but no measurable benefit was found yet.

Fig. 6. Algae based products developed in the Miracles project*. Top row, from left to right: wood panels glued with algae based resins; algae enriched aquafeed pellets; plant protection crates manufactured from algae containing bioplastics. Bottom row, from left to right: prototype facial cream; model food emulsions ("vegan mayonnaise") prepared with algae protein; bioplastic growing pots. See text for details.*

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Cosmetic applications

Partner NATAC has proven that the antioxidant bioactivity of the *I. galbana* extract rich in Fucoxanthin (2.6 %) is 100 times higher than that of pure fucoxanthin, suggesting the presence of other active compounds with antioxidant, stabilizing and/or synergistic properties. NATAC obtained an innovative high added value ingredient based on this extract (IGE-P complex) by encapsulation in phospholipids. Application: dermo-cosmetic products. Two different prototypes - facial serum and facial cream – with potential antiaging and antioxidant properties are available.

Bioactive applications

IMENZ developed protein-based products that were efficient antioxidants and effective biopreservatives, which can be applied to prevent the growth of microorganisms involved in food spoilage. These results will be further exploited and tested for application in food, feed and cosmetics preservation.

Bioplastic applications

RB Biobased Institute and VFT studied the possibility to incorporate microalgae in (biodegradable) thermoplastic compounds and in 2-component (thermo) setting systems. In both systems, microalgae are positively influencing the aesthetics of the end product. In thermoplastic systems, a slow degradation of the resin releases microalgae in the medium, delivering at a slow pace additional functionalities such as ‘fertilising’ the environment (see also ‘fertiliser applications’).

Resin and glue applications

CHIMAR found that microalgae-based biomass can successfully replace 30% of phenol in phenol-formaldehyde resins for gluing plywood panels suited for both interior and exterior applications. Microalgae biomass was also successfully used in bio-based finishing material for wood-based panels.

Fertiliser applications

CE found that different microalgae fractions had a positive influence on plant development and growth, well beyond a normal fertilising effect. They can (1) counteract stress due to drought, poor light and cold; (2) induce formation of flower buds; (3) improve nitrogen uptake in cereals.

Marketing and business plan

To support these application results, a business plan for several scenarios was prepared by VFT. The major target consumer group was identified; its purchase behaviour and perception of microalgae was studied. This led to a new positioning and communication strategy. The finality of this is an increased consumer value for microalgae products. In combination with cost reduction technologies developed within MIRACLES, this may lead to profitable business cases, as calculated in the financial analysis of the different scenarios. Especially multi-product scenarios appeared to be profitable.

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Demonstration of integrated value chains (WP 5)

The aim of WP5 was to demonstrate integrated value chains to deliver proof-of-concept and demonstrate techno-economic viability. The selection of the best performing value chains for validation was based on defined industrial criteria as outlined in weight order: 1) technological readiness for scale-up of the process; 2) feasibility of the process according to the techno-economic approach; 3) impact and market awareness; 4) already finished products approach; 5) multiproduct value chains + co-products: 6) strain diversity, a single one or several involved in the different processes; 7) the conceptual approach, that is, the potential scenarios for the value chains. According to these criteria, validation took place at:

- Pilot scale:
  - synthesis of microalgae-based resins and manufacturing of wood-based panels;
  - manufacturing process of microalgae-enriched aquafeeds incl. product trials;
  - production of oil-enriched microalgal biomass and solvent extraction of oils for food and feed. Data demonstrated that TAG enrichment of microalgae biomass under nitrogen starvation is subjected to uncontrolled parameters (light irradiance, temperature) under culture technology implemented at the Fitoplancton premises. However, technological solutions can allow for correction of such differences;
  - production of thermoplastics containing microalgae for horticulture and other applications;
  - production of microalgae-based thermosetting aesthetic materials.
- Lab-scale:
  - production of a fucoxanthin-enriched extract incl. testing, formulation and development of a prototype for cosmetic applications. The proof of concept, that is the formulation of a facial cream prototype, was adequately delivered on small scale.
  - production of microalgae protein fractions for food applications. The process complexity of protein purification and the low yields were considered in order to decide on the lab-scale of the assay, but final results were successful in terms of rheological and organoleptic properties of the products.

It has to be remarked that scalability of processes demonstrated at lab-scale is technologically feasible. Techno-economic evaluation of biorefinery concepts was also performed during the last year of the project, demonstrating a profitable business potential for three developed multiproduct scenarios.

Techno-economic and sustainability assessment (WP6)

The Techno-Economic Evaluation (TEE) indicates that cultivation is a major cost factor, contributing 60% or more to the total production costs. The actual processing costs are mainly attributed to investment costs and utility and materials costs, depending on the concept under consideration. The analysis also demonstrates a profitable business potential for three developed multi-product biorefinery scenarios.

The further focus was on the environmental and socio-economic performance of the multiproduct biorefinery concepts that are promising options to 1) improve yields of marketable (co)products, 2) reduce waste, and 3) reduce the environmental impact per component. A screening LCA was performed to quantify major environmental impacts including the contribution to Global Warming, Acidification, Eutrophication,

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Photochemical Ozone Formation and Abiotic Depletion (fossil fuels). A hotspot analysis was performed to identify improvement strategies for future optimization.

Cultivation in photobioreactors is a hotspot from environmental perspective, mainly because of the high energy consumption for mixing and cooling of the culture. Implementation of on-site renewable energy production and strategies to save energy can improve this.

Major hotspots in the developed biorefinery concepts include the impact of solvent use (incl. pressurized CO2), heat for drying and electricity consumption. Further improvements in solvent recycling can significantly improve the environmental performance and reduce utility costs. Furthermore, it is realistic to expect that the process steps in the biorefinery can be optimized by enhancing yields and by process integration.

On a product level, algae biomass derived products are not yet competitive with alternatives. This applies in particular to soluble algal proteins and oils compared to conventional agricultural products. This is mainly due to high energy consumption in algae cultivation and processing as well as the early development stage of the used extraction techniques. Further improvements are necessary to make algae a sustainable solution.

An assessment of socio-economic aspects and impacts revealed that algae themselves are not expected to cause significant positive or negative socio-economic impacts. However, algae have significant advantages compared to alternative plant and animal resources incl. fish resources. Especially conventional oil and protein resources have associated problem areas incl. overfishing for fish meal, deforestation and habitat loss in the case of soy and palm oil plantations. Algae can contribute to alleviation of these problems. It is recommended that algae are cultivated on non-arable land to avoid land use change and competition with food crops.

Regarding societal impact, “consumers” are an interesting and powerful stakeholder group, and only limited knowledge about their attitude towards algae is available. Therefore, a screening consumer study was performed by VFT, Unilever and nova to evaluate (1) potential USP as well as the general “image” of algae and (2) perceived concerns which may hinder the acceptance of algae. The study generated useful insights into the consumer perspective of algae-based products and indicates clear trends.

Dissemination and exploitation of results (WP 7)

WP7 is devoted to the dissemination, exploitation and intellectual property management.

The planned dissemination activities in the final year have been carried out successfully. The website and social networks have regularly been updated, two newsletters have been distributed and the project consortium has co-organized the final conference *“Algae Biorefineries for Europe”* in Brussels on 17thand 18thOctober, 2017.

The European summer course on algae biorefinery was held on August 2017 28th to 31st2017 in Wageningen, NL. This 2nd International Course on Microalgae Biorefinery was organized by the VLAG Graduate School in collaboration with Miracles. The course had 20+ participants from 12 nationalities.

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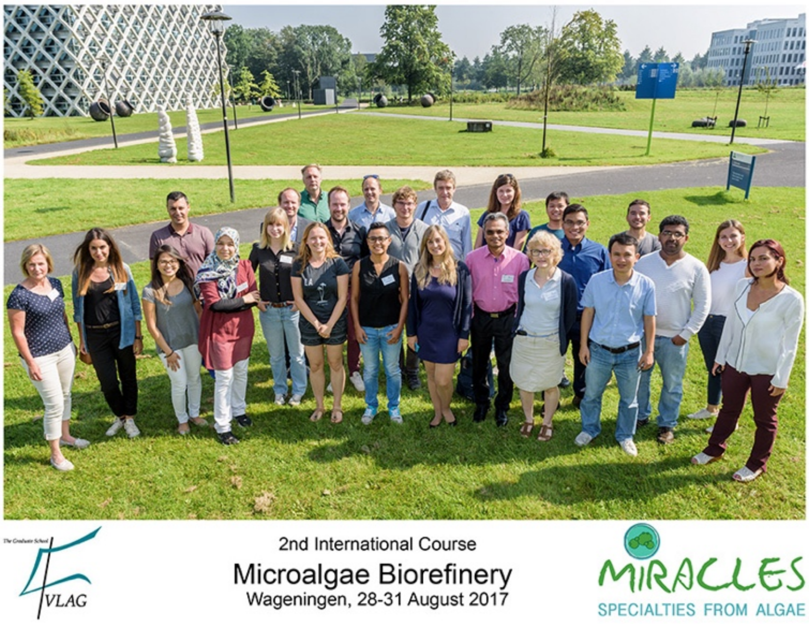
The summer course was a great opportunity to learn about the design of optimal microalgae-based production and biorefinery processes that can be applied for both research and commercial purposes. The course was aimed at academics (postgraduates, PhD students and postdoctoral researchers), as well as professionals from industry. The course was aimed to provide the essential skills for designing optimal microalgae-based biorefineries, from unit operations to the entire process chain, for both research and commercial applications and to be able to address the present bottlenecks in the process chain. In addition, several speakers from industry highlighted the industrial/economic framework of microalgae biorefinery including Miracles partners Carlos Unamunzaga, CEO Fitoplancton, and Philippe Willems, Value For Technology (VFT), and Reza Ranjbar, of AlgaeBiotech.

The course covered the following subjects:

- Description of different process units for each process step in the microalgae biorefinery chain
- Evaluation and optimization of the process units by setting up mass/energy balances for each unit
- Integration of the different process units in a process chain
- Integration of the acquired knowledge into optimal process chains for different business cases with different combinations of end products

MIRACLES Algae Biorefinery Summer School



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Carlos Unamunzaga, CEO Fitoplancton

Philippe Willems, Value For Technology (VFT

Algae Biorefineries for Europe
Towards a sustainable economy

Algae Biorefineries for Europe: Joint Conference held in Brussels, 17-18 October, 2017

In 2013, four major European collaborative R&D projects – Bisigodos, D-Factory, Miracles and PUFA Chain – were launched under the flagship of the European Knowledge-Based Bioeconomy. They were tasked with laying the foundations for a bio-based future built on microalgae: tackling the challenges for capturing CO2 and developing economic, environmentally-sustainable, microalgae-based biorefineries.

The four consortia joined forces to organize the Conference *Algae Biorefineries for Europe: towards a sustainable econom*y to showcase their progress on the sustainable use of microalgae. More than 100 people attended to the conference with representatives from industry, policy makers, NGOs, finance, and researchers across Europe and beyond. Apart from the lectures given by several partners of the consortium, Miracles products and poster were exhibited during the networking sessions.

To download all the conference´s documents incl. lectures, exhibited posters and conference pictures, please visit the website www.algaebiorefineryconference.eu.

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MIRACLES Consortium and Team

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