PUBLISHABLE SUMMARY progress report M19-36

Project context and objectives

Microalgae biotechnology has large potential as a sustainable production platform for food and non-food products. Algae biomass is rich in oils, proteins and carbohydrates and other valuable compounds. For successful scale-up, production costs need to be significantly reduced and (economic) output enhanced. The 4 year MIRACLES project (2013-2017) addresses these challenges by:

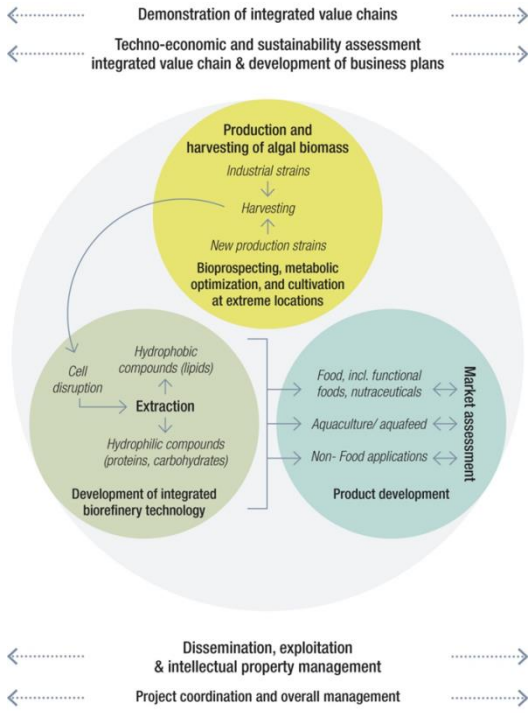

Fig. 1. Overview of project activities

- improving the cost-effectiveness of algae production and processing through technology development along the production chain
- development of multiple-product biorefinery technology for production of specialties from algae
- development of new algae-based products for food, aquaculture and non-food applications

The project activities are presented in *Figure 1.*

The results are evaluated via techno-economic analysis, Life Cycle Assessment and socio-economic analysis of industrial implementation. Business plans for commercialization are developed. The project includes demonstrations to prove techno-economic viability of biorefinery concepts and products.

Progress and main results to date

WP1 partner's activities focus 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) An innovative 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.

Algae biomass (WP1.1 -FITO) from 4 industrial strains (*Isochrysis galbana*, *Nannochloropsis gaditana*, *Phaeodactylum tricornutum* and *Scenedesmus obliquus*) was produced and supplied to partners by Fitoplancton for processing and application R&D. Among the earlier identified indicator genes, a promising gene was found for rapid analysis of the Tri Acyl Glycerides (TAG)-accumulation status in *N. gaditana* cultures under nitrogen deprivation (*Fig. 2A*). Experiments are ongoing to detail the correlation between gene expression and TAG-accumulation to verify/quantify the predicting character of this gene.

Development of technology for CO_2 concentration from the atmosphere (WP1.2 – UT) aims at realizing supported amine based technology for CO_2 capture from air at a suitable concentration for algae cultivation at minimal costs. For several sorbents a good CO_2 adsorption capacity was shown. Co-adsorption of water is an important issue to limit the energy costs for regeneration. The stability of sorbents was investigated under different conditions showing that this is an important parameter for further optimization. Sorbent regeneration studies point towards regeneration at reduced system pressure, at temperatures between 60°C and 120°C with water vapour as condensable purge medium. A novel radial flow contactor is designed, constructed and tested for adsorption performance (*fig 2B*). The results allow for the next development phase: implementing sorbent circulation and regeneration.

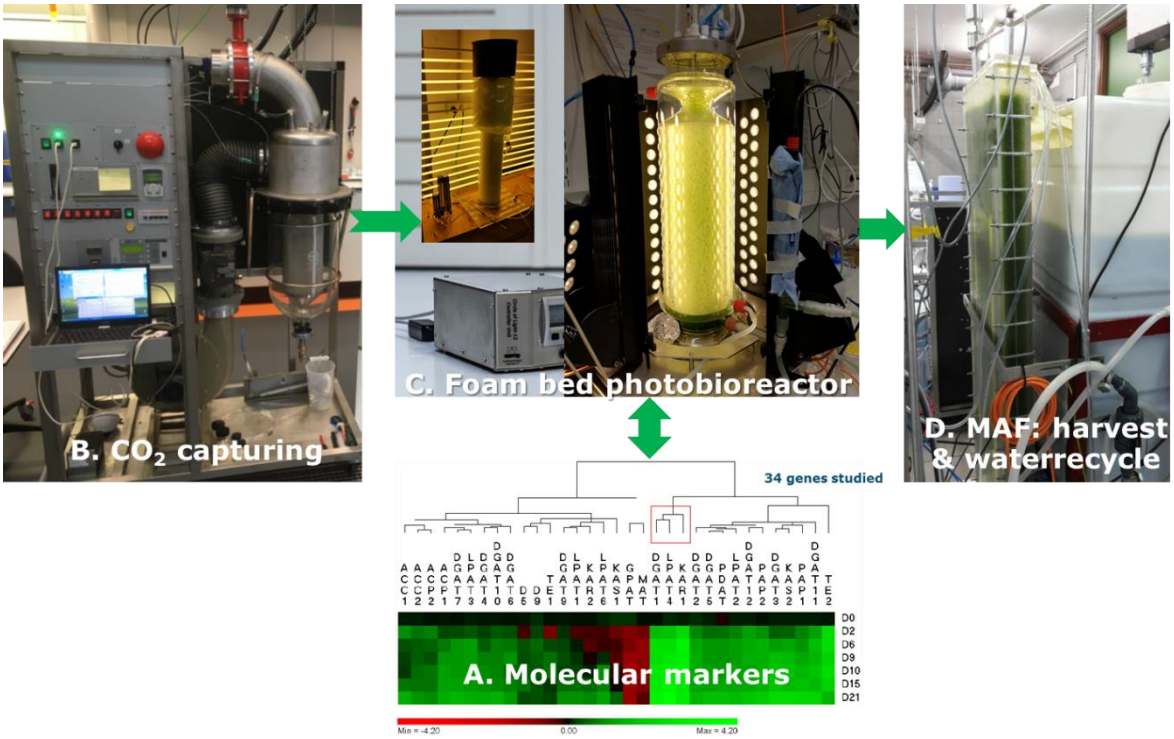

Figure 2: Technology & tools developed for improvement of algae cultivation & harvesting in WP1.

Development of a novel Liquid Foam Bed PhotoBioReactor concept (WP1.3 – WUR & UHU) aims for significant cost reductions due to lower reactor weight and improved gas exchange. A number of surfactants were studied to investigate the toxicity and foaming properties on algae, and Pluronic F68 was selected as the most promising. Good tolerance towards this surfactant was observed for 3 algae species and their fatty acid and pigment content was proven not to be affected. Different reactor types were designed and tested to obtain stable foam conditions for algae growth (*fig 2.C*). A model is developed to describe algae growth in the bioreactor and a techno-economic assessment.

Development of innovative harvesting and medium recycle technologies (WP1.4 – VITO, TMUC) aims to reduce costs and energy use through the development of membrane technology for combined algae harvesting and water and nutrients recycling. After screening tests on lab scale for selection of most suitable membranes and filtration conditions for 4 algae strains, the two most promising concepts were studied in more detail: (1) crossflow on centrate (liquid after

centrifugation) and (2) an innovative integrated pre-harvesting and water recirculation approach using submerged membranes (*fig 2D*). Labscale recirculation tests with *N. gaditana* and *S. obliquus* revealed that 75-90% medium recirculation is possible without negative impacts on algae growth when suitable doses of N and P are added. The integrated algae harvesting & water recycling unit, based on submerged membranes, has been scaled up and improved for performing medium recirculation tests at pilot scale in 300L & 1500L photobioreactors (SUNBUILT facility) in the next project phase.

The objective of **WP2** is to perform **Bioprospecting and selection of robust, highly productive algal species from extreme and diverse climatological conditions.** The aim 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. A sampling strategy was developed taking into consideration how evolutionary pressure in extreme habitats may have developed special strain properties to match identified industrial interests for specific target compounds and new functionalities. To date 254 environmental samples with high diversity have been collected during extensive sampling campaigns in highly diverse climatological conditions (Arctic waters, sub-tropical islands, altiplanic lagoons) over the last 2 years (*Table 1*). This has led to 255 unique clonal isolates of which 70 were screened based on the industrial criteria. So far, 27 candidate strains have been identified with commercial potential based on their high growth potential and high content of PUFA (PolyUnsaturated Fatty Acids), proteins, colorants or specific functionalities.

Table 1. Status of bioprospecting efforts and results from the screening program in MIRACLES WP2

	Environmental samples collected	Enrichments cultures in lab	Cell sorted clonal cells plated	Clonal isolates established	Clonal strains Screened	Candidate strains
FCPCT Sub-tropics, Gran Canarias, Spain	130	260	-	40	10	9
UA Altiplanic lagoon Antofagasta, Chile	69	360	19200	66	20	13
UniRes Arctic/Nordic, Bergen, Norway	55	100	10560	149	40	5
Total	**254**	**720**	**38 400**	**255**	**70**	**27**

To evaluate the productivity potential at different climatic conditions, identical outdoor GWP-III photobioreactor systems have been installed at partners in Norway, Gran Canarias and Chile. Such systems have now been operated in Norway during 2 summer seasons, representing a unique possibility to compare productivity data of reference strains in identical reactor systems under different climatic conditions. Efforts are also made to gain knowledge on how to optimize EPA

(EicosaPentaenoic Acid) productivity through metabolic modelling the fatty acid metabolism of *N. gaditana.* This is done via growth experiments with *N. gaditana* using $NaH^{13}CO_3$ as sole carbon source during nitrogen starvation to study the origin of EPA accumulation in TAG during nitrogen starvation. The metabolism is also studied by transcriptomic monitoring of genes involved in EPA biosynthesis of *N. gaditana* during actual production cycles in outdoor pilot scale photobioreactors, which will increase understanding of EPA metabolism and promote productivity.

WP3 aims at development of integrated biorefinery / processing technologies employing mild disruption, green extraction and fractionation/purification technologies to produce multiple specialty products from microalgae biomass by valorising all biomass components (*Fig. 3*). The focus of recent activities was on (i) further chemical characterization of the four algae strains in use, (ii) knowledge development for mild cell disruption technologies (physical, chemical, enzymatic), (iii) green extraction procedures, (iv) development of fractionation / purification technologies and (v) integration of unit operations of five selected biorefinery chains.

Fig. 3. Process steps in the algae biorefinery

The **biochemical composition** (protein, lipids and their classes, sugars), as well as the presence of pigments of the four algal strains provided has been established in detail (*Fig. 4*)

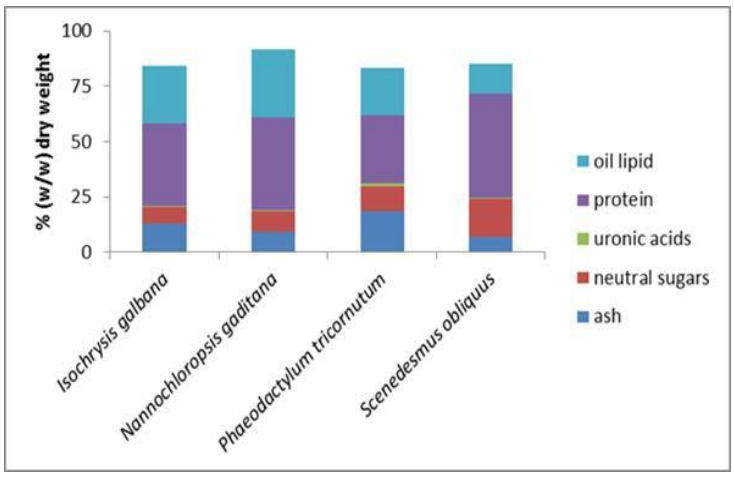

Fig.4. Biochemical composition of used strains

Fig.5. Membrane filtration on lab scale

For **cell disruption**, High-Pressure Homogenization (HPH) and bead milling were efficient enough to release about 50% of proteins with low energy input. Reducing the diameter of the beads caused a higher disintegration efficiency. Enzymatic treatment with proteases showed low energy input and significant release of proteins / peptides but not as high as bead milling and HPH, whereas Pulsed Electric Field (PEF) treatment was least efficient in terms of protein release and energy input for *N. gaditana*. Mild NaOH treatment is an effective and simple procedure for disruption of most algae tested although the maximal amount of protein that could be obtained from *N. gaditana* and *S. obliquus* was restricted to about 50%. In the area of **Green extraction procedures** a sequential

refining extraction procedure, combining compressed fluids technologies, has been developed for *S. obliquus* and *I. galbana.* Furthermore, two novel extraction techniques, pressurized liquid extraction (PLE) and microwave-assisted solvent extraction (MAE), have been evaluated for the recovery of bioactive compounds such as fucoxanthin from *P. tricornutum*. Except *I. galbana,* all studied microalgae required a cell disruption treatment before the extraction train. For **Fractionation and product isolation and purification***, r*esearch focused on the use of membranes to separate and purify proteins (*Fig. 5*). Results show that Increasing the cut off of the membrane does not necessarily improve the filtration process. Optimization of the process is ongoing. Regarding **Integration of unit operations in appropriate biorefinery configurations,** five biorefinery value chains have been selected and designed in SuperPro Designer®.

In the area of **Product Development and Market Assessment (WP4)** the actual application work is ongoing, in line with the planning. In most foreseen application domains, significant progress has been made, leading towards new applications, not yet described in the market. This is an innovation breakthrough.
Several aquafeed formulations containing microalgae are now already available: formulations for juvenile fish, for shrimps and for ornamental fish already demonstrated an interesting potential for microalgae in products with enhanced consumer value *(Fig. 6).* More trials are ongoing / scheduled for seabream, Senegalese sole and salmon. Processing-wise it is possible to formulate aquafeed by direct co-extrusion of algae slurry, eliminating the need for deep dewatering of the algae biomass.

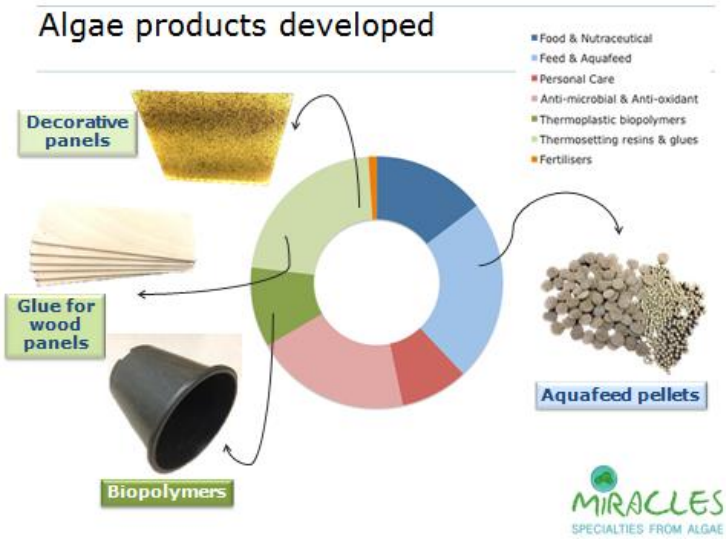

Fig. 6. Algae based products already developed in Miracles.

Innovative specialty products derived from algae have been identified, isolated and evaluated. New anti-microbial peptides can find an application in cosmetics and food systems; novel anti-oxidant molecules are being tested in cosmetics; new colorants are proposed for cosmetics and food. Also in this specialty field, algae derivatives are being validated as plant growth promoter, improving plant quality and aesthetics well beyond a normal fertilising effect.

Even more bulky innovative applications are now demonstrated (*Fig. 6*). Protein-enriched algae extracts (e.g. after oil extraction) can replace up to 30% of phenol in adhesives for plywood. Similar

fractions or whole cell algae are effectively incorporated in aesthetic biomaterials for flooring, table top and light covers and have been compounded with thermoplastic starch to be injection moulded. Products with algae-containing bioplastics are under development.

Food applications are scheduled for the last year of the project, with tests planned with algae oils and proteins in different food systems. Planning for these trials is ready for implementation in 2017.

In support to the application trials, a database with key figures on the market potential for the different applications is ready. Based on this database, a selection of 5 value chains for demonstration in WP5 were retained and business scenarios for each value chain initiated. Next to the raw data acquisition, a lot of attention went towards developing a proper positioning strategy for microalgae products created in MIRACLES.

In conclusion, 3 years into MIRACLES prototypes of different innovative algae-containing end products are available, business cases can be documented and more product development is ongoing.

The overarching aim of the **Demonstration of selected integrated value chains in WP5** is to deliver proof-of-concept and demonstrate techno-economic viability of selected integral process chains for various target products. The scale of the work is adapted to the various biorefinery steps and the required scale for formulation and testing of products by the end-users. Progress to date includes 1) selection of 5 value chains for demonstration with as primary target products Whole cell, Oil & Lipids, Proteins & Carbohydrates, Food Specialties and Non-Food Specialties 2) ongoing pilot scale production of algae batches with optimized composition for demo work by various partners by Fitoplancton. The final aim is to demonstrate four of the best performing integrated value chains.

WP6 aims at **Techno-economic and sustainability assessment of integrated value chains & development of business plans**. Recent work focused on (1) definition of scenarios for the value chains to be evaluated and (2) continued work on conceptual process design models and the initial economic evaluation of the value chains. Scenarios and conceptual process chains (*Fig. 7*) were proposed based on the five product value chains selected for demonstration in WP5 for a deeper investigation (whole cells, broken cells, native soluble proteins, pigments, TAGs).

Together with WP 3 the process integration for the 5 biorefinery chains was performed, using algae strain specific data. Moreover, a first economic evaluation of the biorefinery was performed. The current cost estimates show that for most value chains the cultivation costs are a major contributor to the overall production cost. The cultivation cost represents at least 70% of the total cost, for scenarios whole and broken cells as well as the chain aiming at proteins as the main product. For the pigment value chain the cultivation costs are around 40% of the total cost. For the TAG case, the cultivation costs are two-thirds of the total cost. However, product costs depend strongly on the algae species, due to species specific requirements and yields.

The key conclusion is that generation of co-products and exploitation of residues is vital to 1) improve the economics, 2) reduce waste, and 3) reduce the environmental impact per component.

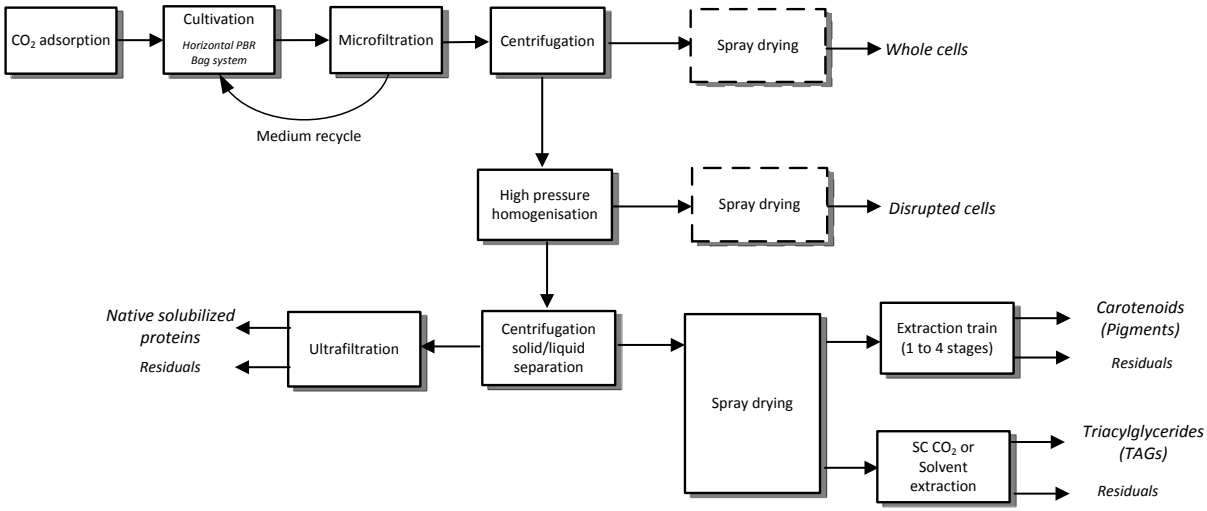

Figure 7: Overview of modelled process chains in WP6.

The final project period will focus on generation of co-products and/or valorisation of residues in order to further develop the process design of the value chains that will be fine-tuned and consolidated in a joint effort of WP3, WP4, WP5 and WP6.

The LCA will address for each value chain different specified key questions and optimization potential. The main reference products are bio-based alternatives. In the downstream processing, drying is a main driver for greenhouse gas emissions. It is a promising option to avoid drying wherever possible. However, further investigations are necessary.

WP7 is devoted to ensure effective **communication, dissemination, exploitation and intellectual property of the project results.** For **internal communication** between the partners the implemented infrastructure (EMDESK platform) to share documents, distribution lists and events organization etc. has shown to be very effective. The **dissemination and exploitation activities** are supported via the project website (more than 21.000 visits to date) and social networks (linked in, twitter and facebook) that are regularly updated. A digital Newsletter is released every 6 months and distributed directly to over 320 stakeholders and also made available on the website. To disseminate results consortium partners participate in conferences incl. co-organisation of the Conference *European Roadmap for an Algae-based industry on 6-8 April 2016, in Olhao, Portugal,* and publish project results in peer reviewed and other journals. On 28 – 31 August 2017 a *Biorefinery summer school* will be organized in collaboration with Wageningen University Graduate School (VLAG). Several partners have collaborated in exchange programs for young researchers for some of the project activities. With regard to **Exploitation and Intellectual Property Management** the consortium follows an agreed procedure to identify and manage potential exploitable results. Several products were presented in the recent consortium meeting in November 2016.

Expected final results and their potential impact and use

The MIRACLES project addresses major hurdles for expansion of the algae industry by enhancing cost-effectiveness of algae production and processing through innovation, development of multiple-product biorefinery technology for specialties from algae and development of new products for food, aquaculture and non-food. Good progress is being made. The project will contribute to bridging the gap to achieve industrial scale production of microalgae and application of derived products through cost reduction, establishing and validating technical and economic data and the development of validated business plans.

The final results of the project will contribute to scale-up and growth of the algae sector within the Bio-Economy and will have a positive impact on the competitiveness and growth of the SME sector. In particular the project will strengthen the competitiveness of the European marine biotechnology industry and by reducing technical bottlenecks in this area making the whole sector more attractive to investments with a positive impact on employment.

Consortium

The MIRACLES consortium has 26 partners including 11 research organizations from 6 EU countries plus Norway and Chile, with complementary expertise. Industrial leadership is guaranteed by the participation of 3 multinational end user companies and 12 SME's

Project website

http://miraclesproject.eu/

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