PROJECT FINAL REPORT

Report Version July 2018

Grant Agreement number: 613588

Project acronym: MIRACLES

Project title: Multi-product Integrated bioRefinery of Algae: from Carbon dioxide and Light Energy to high-value Specialties

Funding Scheme: Collaborative project

Period covered: from 1 November 2013 to 31st October 2017

Name of the scientific representative of the project's co-ordinator, Title and Organisation:

Hans Reith, MSc, Wageningen University

Tel: +31 (0)317 485228 / Mobile +31 6 1285 8852

Fax: -

E-mail: hans.reith@wur.nl

Project website address: http://MIRACLESproject.eu/

List of Abbreviations

ADP	Abiotic Depletion Potential
AP	Acidification Potential
CAPEX	CApital EXpenditures
CF	Concentration Factor
DAC	Direct air capture
DF	DiaFiltration
DHA	(omega-3)Docosa Hexaenoic Acid
DW, DM	Dry Weight, Dry Matter
EPA	(omega 3)EicosaPentaenoic Acid
eq	eguivalent
GWP	Global Warming Potential
GWP	Green Wall Panel (flat panel photobioreactor)
GXL	Gas Expanded Liquid extraction
HPH	High Pressure Homogenisation
(k)Da	(kilo)Dalton
LCA	Life Cycle Assessment
MAF	Membrane(based) Algae Filtration
MF	Microfiltration
MP	Multi Product (biorefinery scenario)
OPEX	OPerating EXpenditures
PBR	PhotoBioReactor
PE	PolyEthylene
PEF	Pulsed Electric Field
PLE	Pressurised Liquid Extraction
sCO2	Supercritical CO_2
SFE	Supercritical Fluid Extraction
SP	Single Product (biorefinery scenario)
TAG	Tri Acyl Glycerides
TLC	ThinLayer Chromatography
UF	UltraFiltration
Y	Yield

Table of Contents

1 Executive summary

MIRACLES is an industry driven R&D and innovation project tasked with the development of economically feasible biorefinery concepts for specialties from microalgae. The project consortium comprises 26 partners with complementary expertise including 11 research organizations, 12 SME's and 3 multinational companies/end users. The project addresses FP7 Work program topic KBBE.2013.3.2-02: The CO_2 algae biorefinery. This 4-year project started in 2013, and ended in 2017.

Microalgae biotechnology has large potential as a production platform for food and non-food products. Successful scale-up requires reduction of production costs and enhancement of the economic output. The project results contribute to this goal by successful development of:

- technological innovations enabling cost reduction in algae production, harvesting and processing;
- multiproduct biorefinery concepts with a profitable business potential;
- a range of new specialty products for application in food, aquaculture and non-food.

By combining cost reduction and value creation MIRACLES contributes towards economically viable microalgae production, demonstrating commercial feasibility and potential profitability of a microalgae venture. The main achievements leading to cost reduction and value creation are:

- a supported amine based technology for CO_2 capture from air for algae growth at competive costs;
- a novel liquid foam bed photobioreactor concept that enables a large reduction of energy use and costs in algae production and harvesting;
- new molecular tools for real-time monitoring and control of TAG and EPA during cultivation of *Nannochloropsis* for cost reduction and enhancement of revenues;
- a submerged membrane system for combined biomass pre-concentration and medium recycling enabling substantial cost savings;
- new, robust production strains for valuable target products with validated outdoor production performance in the tested locations;
- a processing platform (using green solvents) for sequential extraction and fractionation of high added value components from algae biomass for food, pharma and cosmetics;
- knowhow on algae biorefinery and improved technologies for cell disruption, extraction and fractionation, integrated into multiple product biorefinery flowsheets;
- the validation of new high value applications for microalgae in the field of food emulsification and preservation, functional aquafeed, plant growth promotors, cosmetics and biobased materials;
- a positioning strategy based on identified consumer perception, enabling the added value potential of the new applications.

The project has achieved considerable progress beyond the state of the art and a range of exploitable results incl. technologies, new product applications and business models, supported by a marketing and business plan and extensive dissemination and exploitation activities.

MIRACLES technologies are now at a TRL level of 4 and 5, with some exceptions in both directions. This is an increase of at least 2 TRL points compared to the starting position. Further research and demonstration are now needed to validate the technologies at TRL 7-9 and refine the business cases.

The project results were integrated in 8 biorefinery scenarios (5 single product and 3 multiproduct) from biomass production to marketable products incorporating technologies and data developed in the project. The scenarios were evaluated on their costs, profitability and environmental performance (LCA). Reference scale for each biorefinery scenario was a processing capacity of 10,000 Tonne dry algae feedstock /year. The evaluation concludes that

- A 10.000 Tonne multiproduct microalgae biorefinery has an economic potential. The profitability of multiproduct scenarios is caused by new cost reduction technologies developed in MIRACLES combined with value creating new application developments.
- Single product biorefineries of similar sizes however are far from profitable
- Algae production is a dominant cost factor at 60-85% of total costs depending on the scenario.

A screening LCA identified energy use as the major hotspot in microalgae cultivation and refining. Also solvent used in extraction impacts the LCA. The focus in future technology improvement should be on energy saving strategies, cost reduction and enhancing productivity in cultivation (strain improvement, operational adaptation) and processing (yield improvement, process integration).
Comparing LCA data of products (proteins, oils) produced from algae with conventional agricultural alternatives still turns out negative. This is due to the current high energy consumption in algae cultivation and processing and the early development stage of the used extraction techniques. Further improvements and scale up are required to reduce the ecological footprint. Regarding socio-economic impacts, however, algae have significant advantages as 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 at least partial alleviation of these concerns.

A screening consumer study was performed to evaluate (1) potential USP as well as the general “image” of algae and (2) perceived concerns which may hinder acceptance of algae in food, feed and cosmetics. Overall, consumers seem to be open minded and interested in algae products. Concerns are mainly related to off-taste, off-smell and purity (toxins, contaminants). This consumer perspective is most interesting when positioning and communicating about microalgae in the market. All actions and precautions need to be taken to reassure consumers that their concerns are well addressed e.g. via a quality control policy and appropriate communication.

Extensive ongoing dissemination activities coordinated by Dissemination Officer IDC including publications and presentations, website, newsletters, videos and social media postings etc. promote the results of MIRACLES to all stakeholders. Active exploitation of the foreground results is undertaken by the involved industrial partners in collaboration with the projects IPR and Exploitation Officer VFT. Patents are pending and the creation of new commercial ventures is considered.

The final results of the project contribute to growth of the algae sector in the bio-economy and will benefit the European marine biotechnology sector with a positive impact on employment. The results contribute to the EU’s long-term Blue Growth strategy via development of jobs in microalgae biotechnology and the aquaculture sector, and to other EU policies including: Resource-efficient Europe, and Innovating for Sustainable Growth- a Bioeconomy for Europe.

Project website: *http://MIRACLESproject.eu/*

2 Project context and objectives

2.1 Context

Microalgae are a promising feedstock for sustainable production of food, feed and non-food products [1,2,3,] Microalgae can be grown on land unsuitable for agriculture using seawater and CO_2 from flue gas. Algal cultures have a high areal productivity, and the produced biomass is a rich source of proteins, oils, polysaccharides (incl. starch, xylans, pectins, β-glucans) and other high-added value compounds incl. colourants, anti-oxidants, and bioactive ingredients. Microalgae lipids are a potential source of Tri Acyl Glycerides (TAG), phospholipids and glycolipids, and Poly Unsaturated Fatty Acids (PUFA) incl. omega fatty acids EPA and DHA. Algae biorefineries may become advantageous for regions with limited biomass availability, extreme climate conditions and land unsuitable for agriculture incl. desert areas[4] Despite the potential of algae as a production platform the implementation is still limited which is mainly due to unfavourable economics. The costs of algal biomass production need to be reduced and the scale of production needs to be increased significantly. At present algae are being applied in a limited volume (> 10.000 tonne dry weight/year) in various niche markets incl. food supplements, in aquaculture, as a source of PUFA and in personal care products.

Several studies show that even when the cost of biomass production is reduced the algal biomass needs to be refined into multiple products to achieve economic feasibility[5,6,7,8]. The fractionation of algae biomass into multiple products via biorefinery enhances economic revenues per ton of biomass. Thus cost reductions in biomass production and development of effective biorefinery technology and novel products are all essential for the further development and scale-up of the algal sector. Since algae are mostly used in the form of whole biomass, technologies for fractionation are largely unknown. This implies that appropriate technology for multi-product biorefinery of algae is lacking. To develop this technology and a working biorefinery is a major aim of the MIRACLES project.
With regard to cultivation systems the *state-of-the art* is the use of open High Rate Algal Ponds (HRAP) (with severe limitations) and horizontal and vertically stacked tubular photobioreactors (PBR) as well as advanced vertical flat panel reactors. The MIRACLES project focuses on the production of high value specialties and exclusively on semi-closed PBRs as production platform in order to reach optimum process control and product quality.

2.2 Objectives

The overall aim of the MIRACLES project is to develop integrated, multiple-product biorefinery technologies for production of high value specialties from algae for application in food, aquaculture

1 Draaisma, R.B., Wijffels, R.H., Slegers P.M., Brentner, L.B., Roy A., Barbosa M.J. (2012) Food commodities from microalgae, Current Opinion in Biotechnology 2012. http://dx.doi.org/10.1016/j.copbio.2012.09.012
2 Wijffels RH, Barbosa MJ: An outlook on microalgal biofuels. Science 2010, 330:913
3 Milledge JJ: Commercial application of microalgae other than as biofuels. Rev Environ Sci Bio-Technol 2011, 10:31-41.
4 Subhadra, B., Grinson-George. 2010. Algal biorefinery-based industry: an approach to address fuel and food insecurity for a carbon-smart world. J.Sci.Food.Agric. 91: 2-13.
5 Norsker et al. (2011) Microalgal production- a close look at economics, Biotechnology Advances 29: 24-27
6 Wijffels RH, Barbosa MJ, Eppink MHM: Microalgae for the production of bulk chemicals and biofuels. Biofuels Bioproducts Biorefining-Biofpr 2010, 4:287-295.
7 Market opportunities for selected microalgae, Philippe Willems, Value fro Technology, September 2012.
8 Ruiz J., Olivieri G., de Vree J., Bosma R., Willems P., Reith J.H., Eppink M.H.M., Kleinegris D.M.M., Wijffels R.H., Barbosa M.J. (2016) Towards industrial products from microalgae. Energy Environ. Sci., 24, pp.405–413.

and non-food including development of novel products for these markets. Furthermore technologies will be developed for CO_2 concentration from the air for algal growth, optimization of target products in algal biomass and cost reductions in cultivation and harvesting. New industrial strains for extreme locations will be selected via bioprospecting. This will be supported by thorough assessment of market opportunities, techno-economic evaluation, development of integral biorefinery designs and scenarios, and business plans aiming for full valorisation of the algal biomass. To realize successful scale-up, production costs need to be significantly reduced and (economic) output enhanced. The objectives of the MIRACLES project are to address these challenges via

- Improvement of 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 includes demonstrations to prove techno-economic viability of integrated value chains, products and applications.

2.3 Consortium

MIRACLES is an industry driven R&D and innovation project aimed at development of economically feasible biorefinery concepts for production of specialties from microalgae. The project addresses FP7 Work program topic: *KBBE.2013.3.2-02: The CO_2 algae biorefinery.* The consortium has 26 partners including 11 research organizations from 6 EU countries plus Norway and Chile, with complementary expertise ***(Figure 1)***. Strong industrial leadership is guaranteed by the participation of 3 multinational companies active in the target markets and 12 SMEs with activities in algae cultivation, processing, product development, business development and communication. A list of partners incl. partner websites is presented in section 4.6.

Figure 1. *Overview of the MIRACLES consortium*

Detailed objectives

The project consists of 8 Work Packages coordinated by one of the partners with following objectives. The project activities are presented in ***Figure 2***.

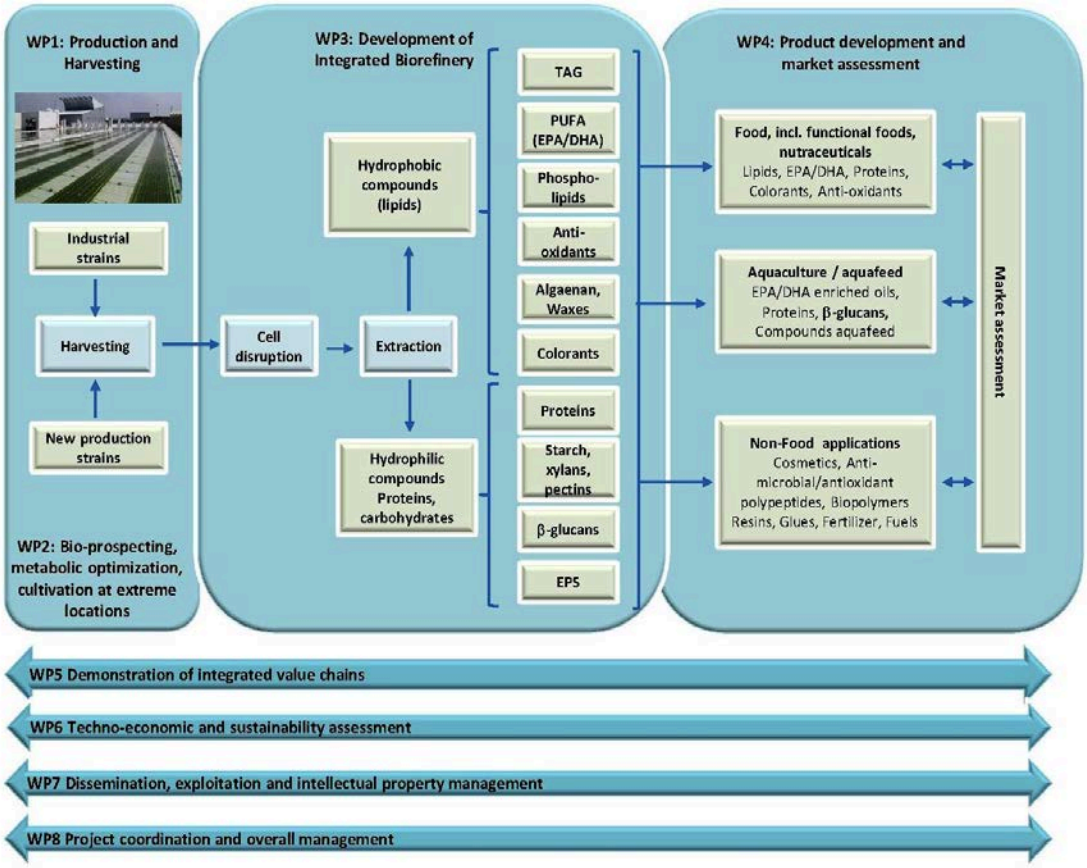

Figure 2 *Concept of integrated algae biorefinery and product development in MIRACLES.*

Work Package 1 (WP 1): Production and harvesting of algal biomass (coordinated by VITO)
Objectives are

- Production of algal biomass for biorefinery and application RTD in WP3 and WP4.
- Development of advanced molecular monitoring tools and strategies to optimize concentration of target biomolecules as a first step in the biorefinery.
- To develop a breakthrough technology for CO2 concentration from the atmosphere to enable cultivation in remote areas such as deserts.
- Development of a novel Liquid Foam Bed flat panel Photo Bio Reactor concept for substantial cost reduction of biomass production
- To achieve water recycle and algae harvesting with membrane technology saving water, nutrients, energy and costs

WP2: Bioprospecting, metabolic optimization and cultivation at extreme locations (coordinated by UniRes)
The main objective of WP2 is to perform bioprospecting and selection of robust, highly productive algal species for extreme climatological conditions to enable cultivation of microalgae in areas with

limited potential for agriculture and in order to broaden the resource base of a growing microalgae industry. Specific objectives are

- To screen the different climatic environments for algal strains with favourable properties
- To perform metabolic modelling and optimization studies to enhance productivity of target products in the algal platform *Nannochloropsis gaditana*
- To optimize the growth and yield of specific compounds by controlling cultivation conditions.
- To evaluate the safety of selected strains
- To produce biomass for further biochemical analysis and valorization of the biomass in WP3.
- To evaluate outdoor production of selected strains under climatic extremes at partner locations

WP3: Development of integrated biorefinery technology (coordinated by WFBR/DLO)
The overarching objective of WP3 is the development of integrated biorefinery / processing technologies employing mild cell wall disruption, green extraction and fractionation/purification technologies to produce multiple specialty products from microalgae biomass by valorising all biomass components. Specific objectives are

- In-depth characterization of algae biomass
- Development of mild cell disruption technologies
- Development of green extraction procedures employing innovative solvents and ScCO2 extraction
- Development of tailor-made enzymes for cell disruption and conversion of biomass fractions
- Development of fractionation/purification technologies via chromatography and/or UF/DF
- Integration of unit operations and optimization of selected biorefinery configurations

WP4: Product development and Market assessment (coordinated by VFT)
The overall objective is to develop applications of algal products produced in WP1, WP2 and WP3 by means of chemical characterization and functionality testing, formulation and performance testing of products on lab and pilot scale and performance of market assessment to validate the proposed applications.

WP5: Demonstration of integrated value chains (coordinated by Fitoplancton)
The principal objective is the demonstration of selected integrated value chain(s) to deliver proof-of-concept and demonstrate techno-economic viability.

WP6: Techno-economic and sustainability assessment integrated value chain & development of business plans (coordinated by NOVA). Major objectives are

- To generate conceptual biorefinery design models as basis for evaluation of the value chain, techno-economic assessment, the LCA and socioeconomic assessment.
- To assess the economics and sustainability of the biorefinery concepts and value chain employing techno-economic evaluation, state-of-the-art Life Cycle Assessment methodology, and socio-economic assessment
- Economic evaluation incl. development of scenarios for multi-product biorefinery value chains for high-value specialties incl. scenarios for co-production of specialties and algal biofuels

- Generation of business cases and business plan

WP7: Dissemination, exploitation & intellectual property management (coordinated by IDC)
Objective: To ensure effective and successful dissemination and exploitation of the project results via

- Effective dissemination and communication with the consortium and external stakeholders
- Training and education activities to contribute to the professional development of those working in the biorefinery and algae sector at various levels with a special focus on SMEs
- Appropiate management and exploitation of Intellectual Property Rights (IPR)

WP8: Project coordination and overall management (coordinated by WU)
Objective: Coordination and Management of the Project, enabling and promoting effective exchange of information and good collaboration to ensure efficient achievement of the project objectives.

This summary report describes the main achieved scientific and technological results, the potential impact and the strategies and plans for dissemination and exploitation of the project results.

3 Main scientific and technological results/foregrounds

3.1 Production and harvesting of algal biomass (WP1)

Contributing partners: VITO (coordinator), WU, UT, UHU, Fitoplancton and TMUC

The major aim of WP1 was to develop innovative technology and tools for a substantial improvement and cost reduction of algae cultivation and harvesting ***(Figure 3***). Additional aim was to produce biomass for the project.

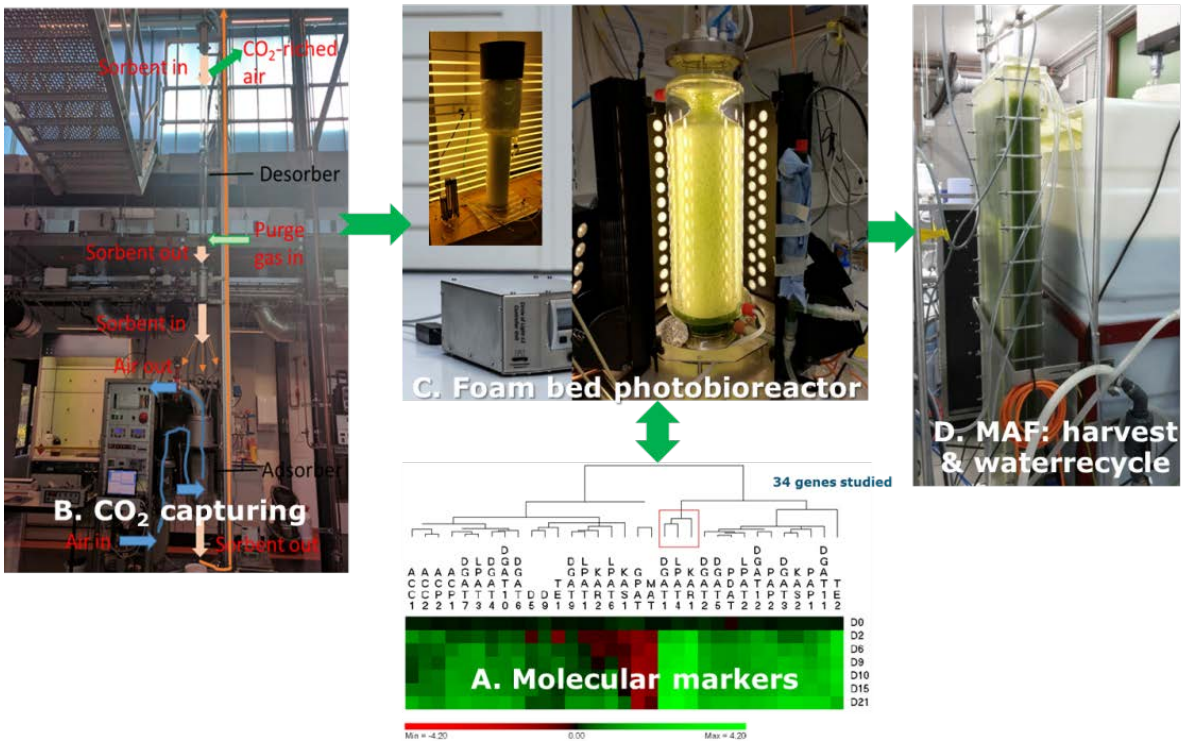

Figure 3. *Technology & tools developed for improvement and cost reduction of algae cultivation and harvesting. A: Molecular markers for real-time monitoring and control of valuable products in the algal biomass; B: Technology for concentration of CO_2 from the atmosphere for algae growth ; C. Photobioreactor concept for algae cultivation in liquid foam ; D. Membrane technology for combined pre-concentration of algae and medium recycling.*

Algae biomass production

The project focused on four major industrial strains produced by partner Fitoplancton i.e. 3 marine strains *Nannochloropsis gaditana*, *Phaeodactylum tricornutum*, *Isochrysis galbana* (T-Iso), and the fresh water strain *Scenedesmus obliquus*. This selection was based on robustness and availability and composition incl. the anticipated level of valuable specialty products. The focus on marine species is justified because they do not require valuable fresh water resources for growth and they are known to produce interesting bio-active compounds in high concentrations. The freshwater species *Scenedesmus obliquus* was added to enhance diversity.

Algae biomass from the 4 industrial strains was produced by partner Fitoplancton **(*Figure 4***) and supplied to project partners for processing and application R&D in various forms (fresh, freeze dried,

disrupted). During the project a number of strains were added based on results from the bioprospecting program. These were cultivated on small scale by the RTD partners UniRes, UiB, FCPCT and UA and supplied to partners for analysis and application R&D.

Figure 4. *Cultivation facilities Fitoplancton Marino S.L. in Cadiz, Spain.*

The pictures show the various cultivation systems incl. indoor photobioreactors (PBR) for pre-cultivation, the pilot plant (top right) and the modular production tubular photobioreactors (left).

The production facility is fully automated and Scada controlled.

Improvement of algae production and development of molecular monitoring tools

Fitoplancton worked on improvements of reactor operation and achieved positive results for growth efficiency and productivity of *Nannochloropsis* in their production units incl. considerable energy saving. Furthermore Fitoplancton prepared a new reactor design using PolyEthylene (PE) bags that enables a further, large reduction of energy consumption. Work on oil/TAG enrichment in *Nannochloropsis* cultures under nitrogen limitation was undertaken in collaboration with WU. In pilot scale runs >2 fold enrichment of TAG was achieved. The data demonstrated that oil enrichment under nitrogen starvation is feasible under strict control of operational parameters esp. temperature.

In general, the productivity of valuable bioactive products in algae biomass can be modified depending on culture conditions incl. nutrient availability, light intensity, or temperature. Such variations are maximized in large scale industrial outdoor cultures, in which development of tools to monitor productivity of bioactives acquires particular relevance. In MIRACLES Fitoplancton successfully developed molecular markers for real time monitoring and control of the productivity of TAG (TriAcylGlycerides) in *N. gaditana* cultures performed under nitrogen deprivation (see ***Figure 3A***). More than 30 genes involved in fatty acid biosynthesis and TAG assembly were analyzed. The relative expression levels of two genes (LPAT4 and DGAT1) correlated well with TAG production and were thus identified as suitable marker genes for rapid analysis of the TAG-accumulation status. The results of growth trials at pilot scale confirmed that these genes are appropriate molecular markers for TAG.
In addition (in WP2) Fitoplancton developed molecular markers to monitor and control the production of the valuable omega-3 fatty acid EPA (EicosaPentaenoicAcid) in *N. gaditana* cultures during a standard production cycle. Expression patterns of genes encoding desaturases and EPA content were compared (***Figure 5***), showing a strong statistical correlation between relative expression ratios of D5/Dw3 and EPA content, thus establishing both genes as suitable molecular markers to control EPA productivity.

	D9	D12	D6	D5	Dw3	EPA
D9		0.976	0.988	0.648	0.709	0.564
D12	0.000		0.988	0.673	0.758	0.552
D6	0.000	0.000		0.661	0.745	0.576
D5	0.049	0.039	0.044		0.842	0.806
Dw3	0.026	0.015	0.017	0.004		0.794
EPA	0.096	0.105	0.088	0.007	0.009	

Spearman Rho

***p*<0.01**

Figure 5. *Correlations between relative gene expression ratios and relative EPA content.*

Both molecular tools constitute a low cost, real-time monitoring technique to enhance revenues via (1) appropriate timing of biomass harvesting to reach optimal TAG and EPA productivity, and (2) use in selection programs for clones with higher capacity and/or efficiency to accumulate TAG and EPA.

Technology for concentration of CO_2 from the atmosphere for algae cultivation

Adsorbent-based CO_2 air capture technology for concentrating CO_2 from ambient air for dosing to algae cultivation was successfully developed by partner UT (See ***Figure 3B***). The main areas of activity included (1) sorbent selection and characterisation; (2) air-sorbent contactor design; (3) sorbent regeneration; (4) lab scale unit: design and operation; (5) process evaluation.

For sorbent selection and characterisation a novel technique, based on combined TG-FTIR analysis and suitable for small samples, was used. As a result, the class of supported amine sorbents seemed most suitable. To enable process development studies and testing at larger scale a commercially available sorbent was selected. Extensive sorbent characterisation with respect to equilibrium capacities for CO_2 adsorption from ambient air and relative humidity (0-95% RH) was performed. The presence of humidity increases the CO_2 capacity by 10-40%. However, for most conditions the water co-adsorption significantly exceeds the CO_2 adsorption capacity, typically by a factor 4-6. Sorbent degradation can be

a significant cost factor. The presence of oxygen above 70°C led to significant sorbent degradation, but at ambient conditions no significant loss of activity was found. With the amine functional group chemically bonded to the (polymeric) backbone of the sorbent, evaporative losses during regeneration could be avoided. For regeneration, several options were explored. For producing pure CO_2 high temperatures (above 100°C), possibly in combination with steam and/or vacuum, are required. However, in this project a cost-effective method using low temperature air stripping was selected to produce a CO_2 enriched air product stream.

Depending on gas-sorbent contacting method, it was found that sorbent saturation can take less than 1 hour up to around 10 hrs. Considering the huge air flow required (min. 1400 m^3 per kg CO_2) a radial flow contactor was designed to minimize pressure drop, while ensuring good sorbent-air contacting and realizing long particle residence times. A successful scale-up strategy was developed and demonstrated, showing consistent results when going from 1 gram to 1.7 kilogram of sorbent, while maintaining a low pressure drop and saturation time. For optimum use of the adsorption contactor, the adsorption and regeneration steps are carried out in different units with sorbent circulation between the units.

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 successfully demonstrated in combination with algae cultivation (***Figure 6).***

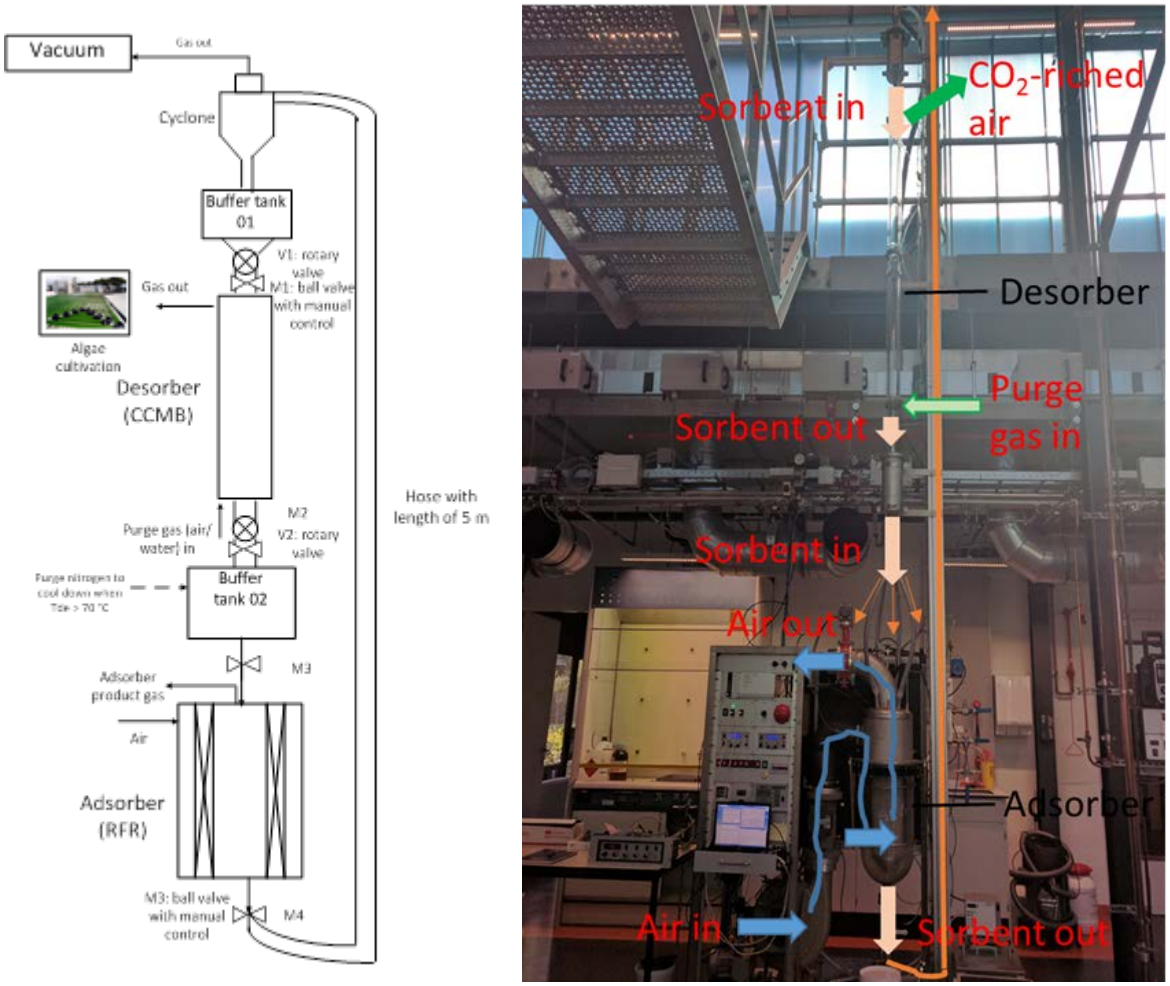

Figure 6. *Left: Flowchart of upgraded pilot plant; Right : photo of the upgraded pilot plant at UT.*

Process evaluation showed that the operating costs for CO_2 production from ambient air are around 75 €/ton CO_2 when producing a CO_2-enriched air stream and around 120 €/ton CO_2 for pure CO_2. Capital costs were not estimated in detail, as they depend strongly on the scale of operation. For the operating costs, the costs for sorbent (-replacement), air-sorbent contacting and desorption of co-adsorbed water are the largest contributors, but also other costs (mainly related to regeneration) like compression, purge gas, sorbent heating etc.) are not negligible. The CO_2 footprint of the process is strongly related to the source of energy used in the process and amounts to less than 0.1 kg of CO_2 per kg of CO_2 captured, based on LCA data for solar PV-based electricity and -heat.

Development of Foam Bed PhotoBioReactor concept

The development of a novel Liquid Foam Bed PhotoBioReactor (LFBPBR) concept by partners WU and UHU aimed at significant reductions in operational costs due to improved gas exchange and high biomass density compared to standard photobioreactors. A proof-of-concept on laboratory scale was successfully achieved.

A number of surfactants were studied to investigate their foaming properties and their toxicity on several species of algae. Regarding the algal species selection, several criteria were experimentally assessed which can be broadly used to evaluate the algal suitability for cultivation in surfactant-based foam: high or moderate foamability of the microalga-surfactant suspension, microalgal partitioning, stability of the foam formed, and robustness and fast growth of the strains. A number of microalgae species were selected. Among the microalgal strains tested, the commercial strains *Chlorella sorokiniana*, *Nannochloropsis gaditana* and *Scenedesmus obliquus* showed the highest potential for cultivation in foam. Regarding the surfactant selection, Pluronic F68 was selected as the most promising surfactant. Good tolerance of this surfactant was observed for 3 algae species and their fatty acid and pigment content was proven not to be affected. Further, it was concluded that (i) microalgae cultivated in surfactant-based stabilized foam are more sensitive to typical stress scenarios for accumulation of carbohydrates and/or lipids (e.g. under Nitrogen limitation); (ii) the surfactant does not seem to play a major role in enhancing lipid extractability from the biomass: cell integrity is not affected and valuable metabolites remain inside the cell until being extracted; (iii) compatible use of fertilizers and surfactant enables cost reduction and ease of operation for microalgae production processes in foam.

Various reactor types were designed and tested to obtain stable foam conditions, and long-term algae growth. In order to compensate for the low algae partitioning to Pluronic F68 stabilized foam, liquid recirculation was introduced into the reactor design. ***Figure 7*** shows the optimized 'generation 3' design of the Foam Bed PhotoBioReactor that was successfully validated on laboratory scale.

The microalgal suspension was continuously pumped from the bottom of the reactor to the top of the foam column where it was allowed to drain down again through the foam. This design allowed for increased mixing and thereby for homogenous algae distribution within the reactor. The volumetric mass transfer coefficient in the foam-bed was 0.14 s^{-1}, revealing that the CO_2 gas transfer rate is an order of magnitude higher compared to bubble column reactors, which enables reduced gas supply requirement compared to conventional liquid-phase photobioreactors. Long-term cultivation (>500 h) of *Chlorella* sp. in a stable foam-bed was achieved. The highest areal productivity of the foam-bed

photobioreactor was 2.4 g m^{-2} h^{-1}, which is lower than maximally achieved in flat panels under similar conditions. This is possibly related to substantial light scattering taking place in the foam leading to a steeper light gradient and increased reflection. Dilution rate and liquid recirculation rate were not optimized yet during our experiments and there is room for further improvement. During continuous reactor operation, biomass densities could be maintained of more than 20 g L^{-1} up to 23 g L^{-1}. This biomass density is 10-fold higher compared to traditional, liquid phase photobioreactors, which thereby contributes to reduced energy requirements for microalgae separation.

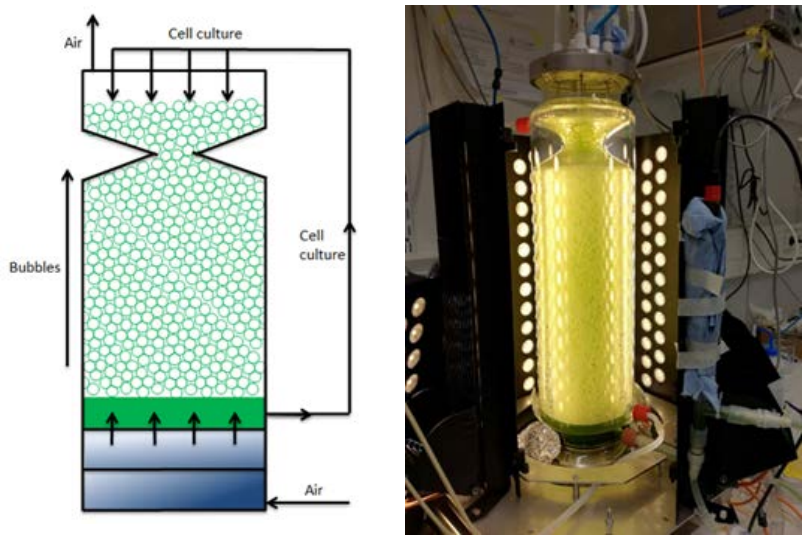

Figure 7 *Process scheme (Left) and lab scale prototype (Right) of optimized 'generation 3' Liquid Foam Bed Photobioreactor.*

A mathematical model was developed to evaluate the potential of liquid foam-bed photobioreactors. The model allowed simulation of the liquid fraction gradient, light penetration, microalgal growth, and gas transfer in foams under different conditions. The simulations for the liquid fraction and the light profile were in a good agreement with experimental data. Model parametric sensitivity was studied for bubble radius, gas flow rate, liquid recirculation rate, light intensity, reactor depth, and biomass density. The model provides insight into the effect of process parameters on areal productivity and energy requirement.

WU developed a conceptual design for a large-scale panel type foam bed photobioreactor. Model simulations based on this design show:

- The energy requirement for the reactor is reduced by an order of magnitude in comparison to a comparable suspension based photobioreactor; this is related to a very large decrease in the required gas flow and the much lower pressure drop in the foam bed.
- The foam bed can run at a biomass density >> 20 g/L or more than one order of magnitude higher than in suspension based systems. This results in a ten-fold reduction in the energy requirement for biomass harvesting.
- Model calculations show that the CO_2 uptake efficiency from the inlet gas was more than 95% resulting in a minimal wasting of carbon dioxide.
- Overall reduction in energy use is ca. ten-fold (reactor operation + harvesting).

The CAPEX of the foam bed reactor is expected to be similar to comparable suspension based PBRs. Cost reductions will thus be achieved mostly in operational costs and via lower costs of harvesting equipment due to the higher biomas density.

The effect of Pluronic F68 on the biochemical composition of microalgae was investigated by UHU in both nutrient replete and nitrogen-starved liquid cultures. Batch cultivation of *Chlorella* in foam was found to lead to increased carbohydrate to lipid ratios.

Membrane technology for combined harvesting and medium recycling

Development of innovative harvesting and medium recycle technologies by partners VITO and TMUC aimed to reduce costs and energy use through the development of membrane technology for algae harvesting and water and nutrients recycling. Four potential approaches were considered (***Figure 8***) besides the reference scenario without medium re-use (*approach A*). A first possibility for reuse of water, is filtration of the medium after the centrifugation step (*approach B*), where dead-end filtration and cross flow filtration were considered. Further, integrated membrane based algae pre-concentration and water recirculation systems were examined (*approach C*) as a more innovative approach. Here, growth medium with algae biomass is filtered first resulting in (1) pre-concentrated algae and (2) permeate that can be recycled. In a subsequent step, the pre-concentrated algae can be further dewatered via centrifugation. Cross flow filtration as well as submerged membrane filtration were considered.

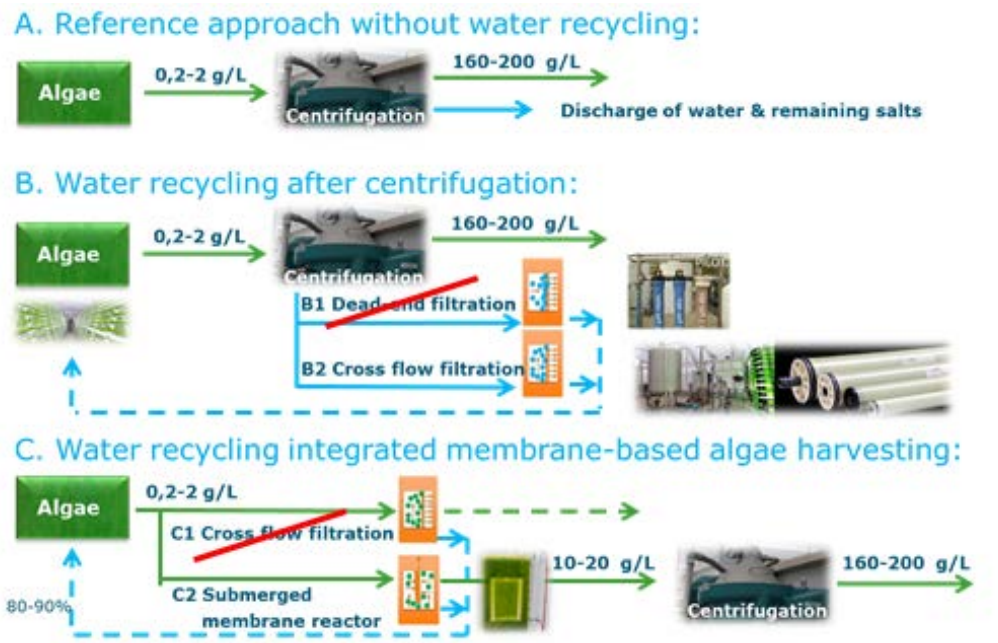

Figure 8. *Schematic overview of different approaches for harvesting and medium recycling.*

In a first research phase, screening tests at lab scale were performed to (1) establish good growth conditions for the 4 algae types and (2) to select the most suitable membranes and filtration conditions for 4 different algae species. In parallel, using *Nannochloropsis gaditana*, the 4 medium recycling approaches were evaluated of which the two most promising concepts were studied more in detail, being: (1) crossflow on centrate (liquid after centrifugation) and (2) an innovative integrated pre-

harvesting and water recirculation approach using submerged membranes prior to the centrifugation step. Based on lab & pilot trials, both approaches were proven to be technically possible. However, based on a first iteration of a techno-economic assessment, the innovative submerged membrane based technology (MAF-technology, approach C2) was found to be economically more favourable. The required reduced capacity of the centrifuge compensated by far the extra cost for the MAF device.

The impact of medium recycling on algae growth was evaluated by algae growth tests as well as chemical analyses on the permeate. Lab-scale medium recirculation tests with *Nannochloropsis gaditana* and *Scenedesmus obliquus* revealed that 75-90% medium recirculation is possible without negative impacts on algae growth when suitable doses of N and P are added. Chemical analyses confirm that most medium compounds (> 99.8 %) do pass the membrane and can be recycled.

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 (***Figure 9***).

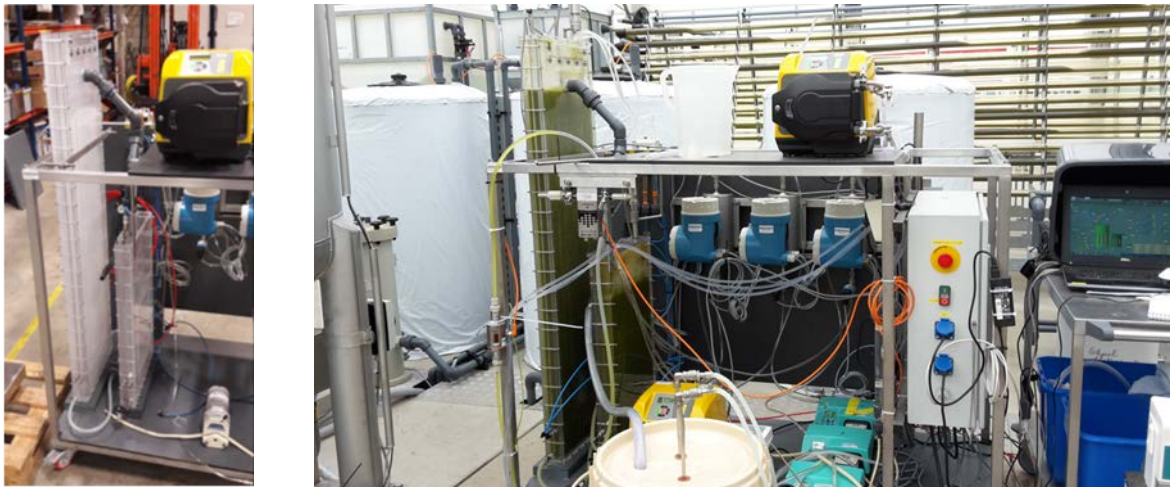

Figure 9. *Continuous MAF-device (LEFT) integrated in the Sunbuilt algae pilot plant (RIGHT) at the TMC facilities.*

In a long term continuous pilot scale growth trial (>40 days) medium recoveries of 90-95 % (on average 92%) could be obtained. The fluxes were inversely related with the algae density ranging between 9 - 70 L/m²h. Volume concentration factors of >40 were reached, with measured final algae concentration up to 34 g DW/kg. Membrane fouling occurred during longer term filtrations but was shown to be reversible. The MAF-systems were proven to function under batch as well as feed & bleed operational conditions, including long-term operation directly linked to a photobioreactor. Via turbidity measurements, the MAF-software was able to link algae harvesting volumes directly to algae growth rates, which offers potential to keep the algae biomass in the exponential growth phase. Recycling of media during *N. gaditana* cultivation using the MAF-technology has minor or no impact on microalgae growth. Addition of nitrogen, phosphorus and small amounts of complete medium are sufficient to maintain normal growth rates and algal densities.

It can be concluded that the MAF-technology allows a considerable reduction of the eco-footprint compared to the reference case without medium recycling. Simulations and calculations were performed for a 10 ha production plant to assess the potential benefits. The achieved medium recycling rate (average 92%) would imply a reduction of water consumption to ca. 12% in a simulated 10 ha system, while the net use of minerals can be reduced by a factor > 7. Results further show that use of the MAF-technology reduces energy consumption to ca. 25% compared to the reference case without water re-use (based on 10 ha simulation case for *Nannochloropsis gaditana*). For a 10 ha cultivation system with MAF-technology it was calculated that the CAPEX decreases slightly (till 87%) compared to the reference case. Regarding operational costs, use of the MAF-technology will reduce costs of energy, water, minerals, water discharge considerably (till 14%) compared to the reference case.

3.2 Bioprospecting and strain selection from microalgae under climatological extremes in Norway, Spain and Chile (WP2)

Contributing partners: UniRes (Coordinator), WU, FCPCT, UiB, UA, FITO, URDV.

Bioprospecting and screening of strains

The first task focussed on bioprospecting and strain selection from microalgae under climatological extremes in Norway, Spain, and Chile by partners UniRes and UiB, FCPCT and UA. Aim was to find robust, highly productive algal species with appropriate biomass characteristics at extreme climatological conditions through a fit-for-purpose bioprospecting and strain selection program. First, an inventory of available resources was finalised, and complete lists of strains and isolates from in-house collections were collected at each bioprospecting partner. Moreover, standard protocols for sampling, isolation, cultivation, identification and characterization of strains were exchanged between partners. To ensure that the selected strains would fit the specified needs of the industry partners, benchmark levels and criteria were set, both for biomass productivities and content of interesting components. Focus components were: total lipids, poly-unsaturated fatty acids (PUFA) content and composition, proteins, polysaccharides, specifically bioactive biomolecules such as β-glucans, carotenoids (as pigments and antioxidants) and polyphenols.

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**

Next, environmental samples were gathered at each extreme location. UniRes, in collaboration with UiB, searched for strains with high PUFA content, while bioprospecting existing and novel microalgae cultures from the temperate and sub-arctic Norwegian fjords, coastal and arctic oceanic waters. FCPCT has used the Canary Islands, volcanic oceanic islands that harbour diverse climatic conditions, pristine ecosystems and habitats that require extreme adaptation of biological properties, for their bioprospecting. In Chile, partner UA has found interesting microalgae in biological samples from the marine and freshwater habitats in the Atacama Desert and the Antofagasta shoreline.
From the environmental samples, strains were selected and clonal cultures of the most promising isolates with high growth potential were developed. Isolates were screened for high lipid, PUFA content and composition, β-glucans, polysaccharide, antioxidants and proteins. Specific, promising isolates were further characterized on laboratory scale with regard to productivity and biochemical composition to evaluate commercial potential in the application areas ***(Table 1).*** This resulted in 32 candidate strains for further exploration.

Evaluation of biomass production in pilot scale outdoor photobioreactors

The biomass production of selected 'candidate' strains has been evaluated in pilot-scale outdoor photobioreactors (Green Wall Panel; GWP) under the climatological conditions at the respective partner locations *(**Figure 10**).* The different climates and varying environmental conditions have been closely monitored during all experiments, and were evaluated in relation to growth and biochemical profile of the selected strains.

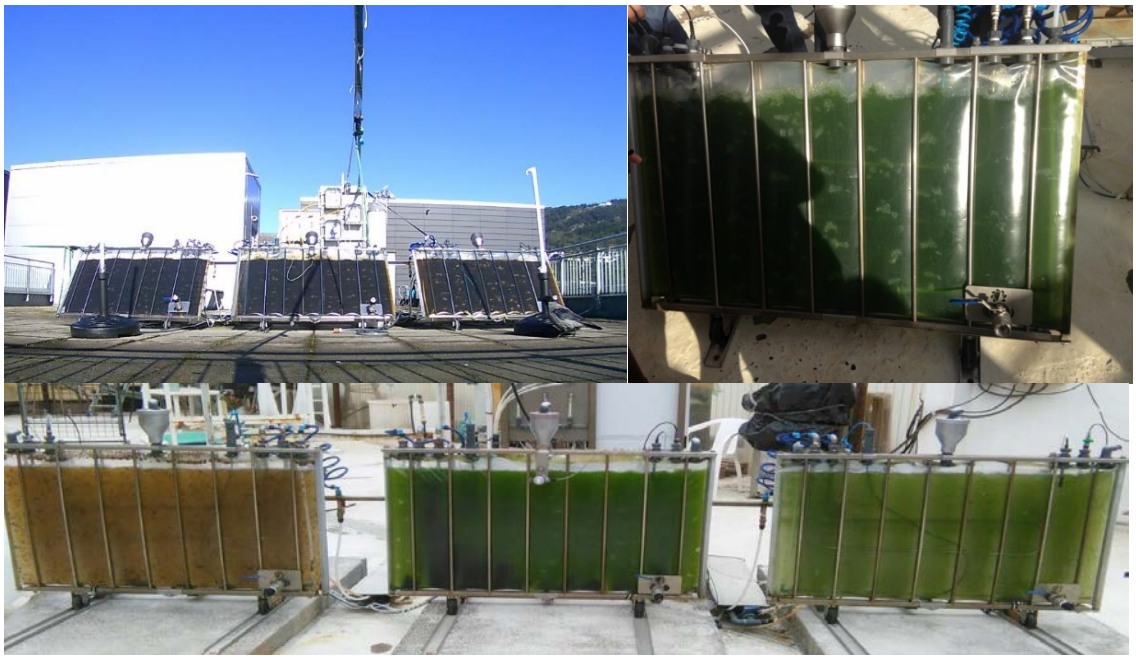

Figure 10. *The different pilotscale photobioreactor set-ups at Bergen, Norway (upper left), Mejillones, Chile (upper right), and Gran Canaria, Spain (bottom).*

Location Bergen, Norway

Outdoor production of five different strains was feasible during at least six months of the year at the climate conditions in Bergen, western Norway. However, when related to studies from lower latitudes

these productivities were comparatively low, most likely due to the reduced irradiances at the given location and as yet to be optimized reactor operation. From the eight selected promising, candidate strains from Norway, five were scaled-up to outdoor production tests. The three locally isolated strains of *Phaeodactylum tricornutum* (B58, M28 and M29) and one *Entomoneis* sp. strain (M122) grew well under outdoor conditions. These strains were compared to the industrial *P. tricornutum* strain provided by partner Fitoplancton. The *Attheya* strain appeared not to grow on the richer growth medium and the higher temperatures reached outdoors, and neither did the selected *Thalassiosira* (diatom) strain show successful growth during the outdoor experiments. Both B58 and M29 are considered to be potentially interesting strains with high biomass productivity and an interesting biomass composition (especially EPA content), although they never outperformed the industrial Fitoplancton strain under outdoor conditions in Norway.

Location Gran Canaria, Spain

The outdoor experiments in Gran Canaria indicate that all selected strains 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-glucan in the palmella stage. ß-glucans are polysaccharides with a high medicinal and economic potential.

Location Antofagasta, Chile

From the bioprospected strains in the Atacama desert and along the coast of Antofagasta that were deemed potentially interesting, 4 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 Of all these strains, the *N. draveillensis* strain 53.3 diatom showed the highest growth in the GWP. Combined with its high content of fucoxanthin and EPA, which complement its antioxidant activity, this strain has high potential for the food industry.

The overall results from the bioprospecting, screening, laboratory characterization and outdoors validation program at the different locations are summarized here:

- 58 environmental samples have been collected from Arctic and Nordic fjords, forming the starting point of the screening program. From this, 8 strains have been identified with high growth rates and high EPA content compared to the benchmark levels..
- A promising strain of the diatom *Attheya septentrionalis* showed 6.4% EPA content of total DW in the stationary phase. Strains grown at lower salinity (22 ppm) even had 7.1% EPA content per total DW after 5 days stationary phase.
- Three *P. tricornutum* strains (M28, M29 and B58) have been successfully grown outdoors in the flat-panel photobioreactors. In addition, the *Entomoneis* strain M122 has shown to be very interesting for production of PUFAs outdoors. Achieved EPA productivity by the local strains was only slightly lower than the reference *Phaeodactylum* strain supplied by Fitoplancton. Process optimization can increase these productivity levels.
- 213 environmental samples have been collected from Saltworks, Marshlands and other special ecosystems in the Canary Islands, forming the starting point of the screening program. This led to 24 candidate strains that were screened, particularly for β-glucans, polysaccharide,

antioxidants and proteins. Nine of these strains showed high growth rates and high contents of target products.

- Five selected strains have demonstrated a potential high content of the different metabolites (high in either proteins, carbohydrates, β-glucans, polyphenols or anti-oxidant activity) studied under outdoor conditions in pilot-scale photobioreactors.
- Especially *Euglenas* are a good candidate to produce at large scale because of the high amount of insoluble ß-glucan produced in the palmella stage.
- 69 environmental samples have been collected from altiplanic lagoons in the Atacama Desert, salt lakes, the west coast of Chile and other special ecosystems, forming the starting point of the screening program. Of the 66 clonal strains established, 20 strains have been screened for biomass productivity and the content of polysaccharide, antioxidants, pigments and proteins.
- 15 candidate strains, with high growth rates and high target product contents, have been selected for further characterization. Four strains grew under outdoor conditions in the pilot photobioreactors. These were: *M. inermun* (proteins); *M. inermun* (carbohydrates); *Chlorella* sp. (proteins); and last, the *Nitzschia* strain (fucoxanthin). *Oscillatoria* sp. (phycocyanin) and strain 18.2 (phycoerythin) grew only in indoor conditions.
- All selected strains from the Nordic climate, and the subtropical climate showed similar yields on light. Process optimization is needed to reach higher photosynthetic efficiencies.

Overall, the program resulted in a number of new, robust strains for production of valuable ingredients for the target markets with validated outdoor production in the tested locations.

Metabolic modelling and studies to maximize the yields of valuable products

Next to selecting better strains from environmental samples, work has been done to better understand the lipid metabolism of the microalgae *Nannochloropsis gaditana* with regard to fatty acid accumulation in both TAG and polar membrane lipids during nitrogen starvation, with special interest in the omega-3 fatty acid EicosaPentaenoicAcid (EPA). Responses and genetic regulation in *N. gaditana* under nitrogen-starved conditions involving fatty acid synthesis and TAG accumulation were studied using a time-evolved transcriptional profiling experiment and were compared to nitrogen replete conditions.

On the transcriptional level a major change is observed in the flow of carbon to provide precursors for fatty acid synthesis. The regulation of TAG synthesis is complex, not pointing to the up- or downregulation of the whole pathway. Shuttling of (photosynthetic) membrane lipids toward TAG may play a more important role very early (< 20 h) than later in the nitrogen starvation phase, as the only identified PDAT gene was downregulated from 20 h onward. The genes involved in *de novo* fatty acid synthesis are mostly downregulated, which is surprising in the light of the observed large increase in lipids. However, this may be caused by a feedback mechanism due to the increased fatty acid precursors.

The increase of 16:1 and 18:1 fatty acids relative abundance can be explained on a transcriptional level. Three delta-9 desaturases, catalysing the first desaturation step from 16:0 to 16:1 and 18:0 to 18:1 were consistently upregulated in the nitrogen starvation phase. By using ^{13}C carbon *de novo* synthesis,

turnover of fatty acids and transport between the different lipid fractions were distinguished for the different fatty acids. Palmitic acid, palmitoleic acid and oleic acid in TAG were shown to be mainly made *de novo* and from reshuffling of carbon within the cell during nitrogen starvation. For eicosapentaenoic acid (EPA) the transport from the polar membrane lipids to TAG was shown and *de novo* synthesized during nitrogen starvation. 20:5 (EPA) was produced during the whole nitrogen starvation phase and partially transferred from the PL to the TAG fraction. The acquired results and insights form a good basis for further development to improve production of lipids and EPA using *Nannochloropsis.*

Production of biomass for biorefinery RTD and initial safety assessment

A final task in WP2 was to produce biomass of interesting strains and supply this biomass to partners for characterization and application testing. In total >12 kg frozen paste and >0.4 kg of freeze-dried material of 17 different strains have been produced and distributed for analysis (a.o. pigments, biochemical characterization), cell disruption and homogenization testing, and application testing for: synthesis of resins and adhesives, proteins for bioactive properties and fermentation, fertilizer capacity, and functionality of use in biomaterials.

For three microalgae: *Scenedesmus obliquus*, *Nannochloropsis* sp. and *Isochrysis galbana* used within MIRACLES an initial (food) safety assessment has been performed by partner URDV in which available (toxicity) data and data gaps were identified.

3.3 Development of integrated biorefinery technology (WP3)

In WP3 we aimed 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 11)*** for application in food, aquaculture and several non-food purposes. Main contributing partners: WFBR/DLO (coordinator), WU, CSIC, DNL, FITO, IMENZ, ET, DSM.

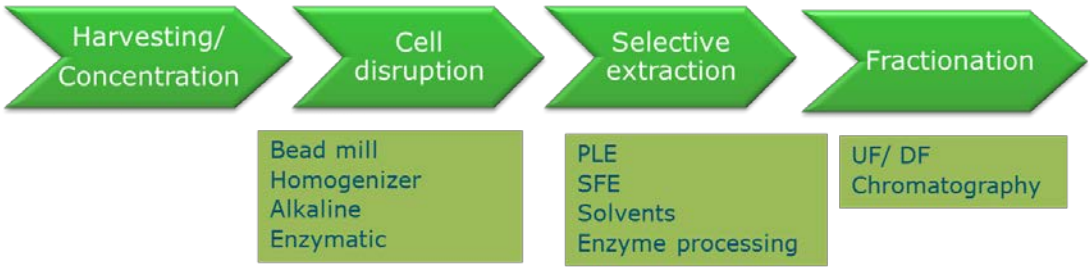

Figure 11. *Process steps in the algae biorefinery*

Characterization of biomass composition

For extraction of valuable compounds it is of importance to know the composition of the biomass which may vary depending on species, strain and cultivation conditions. The four industrial algal strains

used in MIRACLES were characterized. In addition, the chemical composition of strains from different climatic environments isolated by different partners were determined.

Protein was the main fraction in all species investigated followed by lipid/oil, ash and sugar fractions. *S. obliquus* contained more sugars than lipid/oil and ash. *I. galbana* contained the highest amount of the omega-3 fatty acid docosahexanenoic acid (DHA) and *N. gaditana* and *P. tricornutum* the highest amount of omega-3 eicosapentaenoic acid (EPA). Galactose and glucose are the most abundant sugars in all four species investigated. Mannose was also present in higher amounts in *S. obliquus* and *P. tricornutum*. In total about 77-90% (w/w) dry weight of the total compounds was identified (***Figure* 12**). Different lipid classes, such as glycolipids, phospholipids, sterols, pigments, were identified in the oil/lipid extracts by the use of Thin Layer Chromatography (TLC).

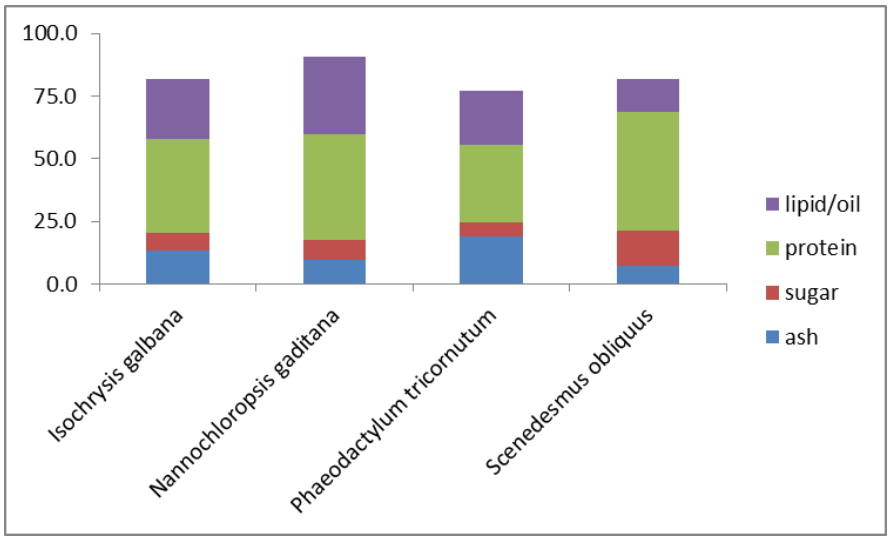

***Figure 12**. Ash, sugar, protein, lipid/oil content of different microalgae based on dry weight % (w/w).*

In order to enhance the oil content in *Nannochloropsis*, for application in food or fish feed, several batches were grown by Fitoplancton on pilot scale under N-starvation to enhance the oil/TAG concentration. Batch LO1 of this stressed *N. gaditana* showed a high TAG-content, whereas batches LO2 and LO3 showed a much lower TAG content. In all batches the EPA content was low. The sugar composition revealed that the glucose content was increased in stressed *N. gaditana*, whereas galactose and mannose content was decreased.

Four strains resulting from the bioprospecting, screening and selection program were biochemically characterized. Different batches of *Chlamydomonas* sp. *Chrysoreinhardia* sp., *Euglena* sp. and one batch of *P. tricornutum* B58 were analysed. *P. tricornutum* B58 contained relatively high amounts of EPA, *Euglena* sp. contained mainly glucose as carbohydrate.

Cell disruption

Mechanical treatment

Several cell disruption methods were tested on *Nannochloropsis gaditana*, to evaluate their efficiency in terms of cell disintegration, energy input and release of soluble proteins. High-pressure homogenization (HPH) and bead milling were the most efficient with >95% cell disintegration, ±50% (w/w) release of total proteins and low energy input (<0.5 $kWh.kg^{-1}_{biomass}$). Enzymatic treatment

required low energy input (<0.34 $kWh.kg^{-1}_{biomass}$), but it only released ±35% protein (w/w). Pulsed Electric Field (PEF) was neither energy-efficient (10.44 $kWh.kg^{-1}_{biomass}$) nor successful for protein release (only 10% proteins w/w) and cell disintegration in the strain tested. The release of proteins after applying HPH and bead milling always required less intensive operating conditions for cell disruption. The energy cost per unit of released protein ranged from 0.15–0.25 $€.kg_{Protein}^{-1}$ in case of HPH, and up to 2–20 $€.kg_{Protein}^{-1}$ in case of PEF (***Figure 13***. The study confirmed the possibility of performing an efficient cell disruption of *N. gaditana* by bead milling or HPH, with low energy input.[9] The study showed that PEF was neither energy-efficient, nor an appropriate method to break the tough cell wall of *N. gaditana* to release soluble proteins. This low efficiency for protein liberation does not entail that PEF is not efficient to release other components, or to disintegrate weak cell walled species. The protein release after mechanical treatments was ~50%, and supplementary measures could be considered such as combining high pH or enzymes with the mechanical treatments already tested.

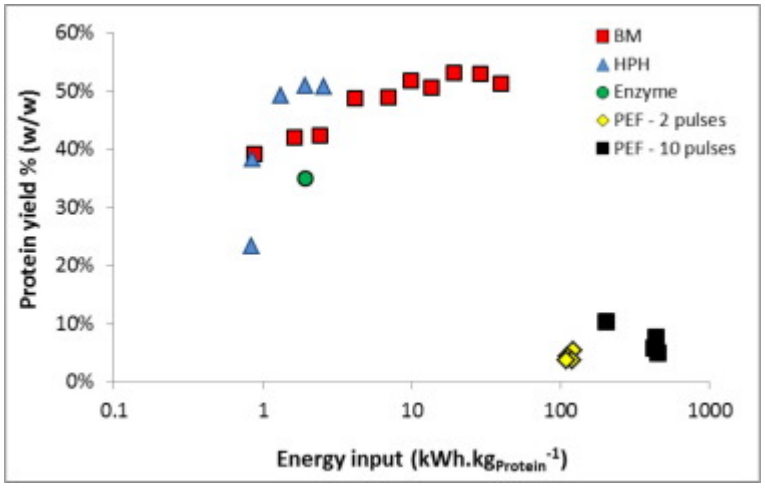

Figure 13 *Overall comparison between the cell disruption methods tested with Nannochloropsis gaditana. The results displayed represent the protein yield and the specific energy input (E). BM is bead milling, HPH is high pressure homogenization, PEF is pulsed electric field. (from Safi et al, 2017)*

Chemical treatment

In addition to mechanical treatment, also chemical treatment, e,g, incubation of algal biomass at elevated temperatures and high pH (pH 12) was efficient in cell disruption. Such a pre-treatment method, however is limited to applications that do not require protein functionality. For peptide formation, after enzymatic treatment it can be quite efficient.

Enzymatic treatment

Protease treatment of algal biomass resulted in efficient cell disruption and about 30-40 % release of proteins/peptides (w/w). For *N. gaditana* >70% of proteins that were released after high pressure homogenization were fractionated after protease treatment and membrane filtration. Without protease treatment only 40% was recovered after filtration.

9 Safi C, Cabas Rodriguez L, Mulder WJ, Engelen-Smit N, Spekking W, Van den Broek LAM, Olivieri G, Sijtsma L (2017) Energy consumption and water-soluble protein release by cell wall disruption of Nannochloropsis gaditana. Bioresour Tech 239:204-210. https://doi.org/10.1016/j.biortech.2017.05.012.

In general, cell wall degrading enzymes that are known to act on land based plant material were not or only limited active towards the algal biomass tested. In order to identify and develop new enzymes, diverse sources including bacteria, (marine) fungi and algae were screened for interference with algal growth and cell wall disintegration. Combinations of enzymes from different sources can be applied to improve the disruption of algae. For each algal species, however, the most effective combination of enzymes needs to be prepared based on the unique properties of the enzymes with respect to optimal activity and substrate specificity. Nevertheless, additional research, in particular related to the production, purification and characterization of the different candidate enzymes will be essential, prior to application.

In addition, a very large number of records is available of DNA sequences associated with the (potential) breakdown of algal cell wall polymers. Further studies into these records may pinpoint more specific enzymes that may be applicable for the extraction of metabolites from algae.

Extraction and fractionation

Lipid extraction

Supercritical CO_2 (ScCO2) was explored to obtain high lipid yield from intact and/or HPH disrupted algae. For the ScCO2 extraction two sequential extraction steps were performed: 1) extraction with neat CO_2 (extremely non-polar) to extract highly lipophilic compounds and 2) extraction with CO_2 and ethanol as co-solvent to obtain extracts rich in other bioactives (more polar). Cell disruption, e.g. by homogenisation, was important for oil extraction and resulted in higher yields although the overall oil extraction yield was quite low. For practical reasons, to produce sufficient material (kg scale) for application research in WP4 lipids were extracted with two different solvent systems: (i) hexane/isopropanol (IPA) (ratio 3/2) and (ii) hexane/ethanol (EtOH) (ratio 92/8). High lipid yields were obtained for *Phaeodactylum* with both solvent systems. Hexane/isopropanol (IPA) is preferred to extract *Nannochloropsis* and *Scenedesmus* while hexane/ethanol (EtOH) is more efficient for the extraction of lipids from *Isochrysis.* The HPH pretreatment had a pronounced effect on extraction efficiency. By using organic solvents the lipid yields were considerably higher as compared to the used ScCO2 and almost 100% efficiency was obtained.

Ecotreasures undertook efforts to refine the oil with bleaching earth, calcium bentonite (TONSIL 210 FF), which possesses an outstanding adsorptive capacity for polar compounds like chlorophyll, carotenoids, phospholipids, peroxides, via chemisorption. High amounts of Tonsil 210 FF were required to remove chlorophyll from the oil. When e.g. 400 grams of Tonsil was added to 50 grams of oil, a dark red coloured oil was obtained. The amount of chlorophyll and carotenoids present in the oil decreased from initially 40.8 mg/g chlorophyll and 29.5 mg/g carotenoids to respectively 0.056 mg/g chlorophyll and 2.12 mg/g carotenoids after refining and 30% of the oil was recovered *(**Figure 14**).*

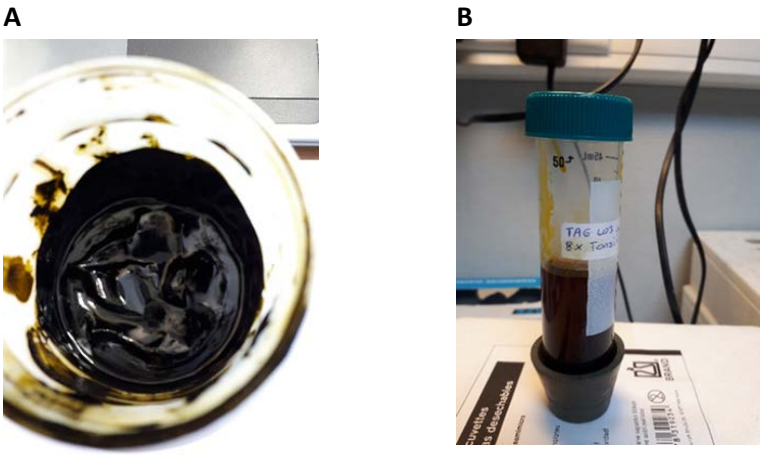

Figure 14. *Oil extracted from Nannochloropsis A. before Tonsil treatment and B. after Tonsil treatment.*

The fatty acids profile of the refined oil did not differ much from the fatty acids profile of the crude oil. Since algae contain interesting carotenoids such as Zeaxanthin and Fucoxanthin, the Tonsil residues after treatment of the oil were extracted by partner CSIC. Indeed, it was possible to recover up to 88% of the carotenoids.

Protein fractionation

A mild biorefinery process was investigated for the microalga *Nannochloropsis gaditana*, to obtain an enriched fraction of water soluble proteins free from chlorophyll. After harvesting, a 100 g.L−1 solution of cells was first subjected to cell disruption by either high-pressure homogenization (HPH) or enzymatic treatment. HPH resulted in a larger release of proteins (49%) in the aqueous phase compared to the Alcalase incubation (35%). In both cases, an ultrafiltration/diafiltration step (UF/DF) was then performed on the supernatant obtained from cell disruption by testing different membrane cut-off (1000 kDa, 500 kDa and 300 kDa). After optimising the process conditions, the combination of enzymatic treatment followed by UF/DF resulted in a larger overall yield of water soluble proteins (24.8%) in the permeate compared to the combination of HPH followed by UF/DF (17.4%). A gel polarization model was implemented to assess the maximum achievable concentration factor during ultrafiltration and the mass transfer coefficient related to the theoretical permeation flux rate[10]

Extraction of colorants and bioactives

A downstream processing platform using various pressurized green solvents has been developed to extract bioactive compounds from the microalga *Isochrysis galbana*. Extractions were performed in four sequential steps using (1) supercritical CO_2 ($ScCO_2$), (2) $ScCO_2$/ethanol (Gas Expanded Liquid, GXL), (3) pure ethanol, and (4) pure water as solvents, respectively ***(Figure 15 Top***). The residue of each extraction step was used as the raw material for the next extraction. Optimization of the $ScCO_2$ extraction was performed by factorial design in order to maximize carotenoid extraction. During the second step, different percentages of ethanol were evaluated (15%, 45% and 75%) in order to maximize the extraction yield of fucoxanthin, the main carotenoid present in this alga.

[10] Safi C, Olivieri G, Campos RP, Engelen-Smit N, Mulder WJ, Van den Broek LAM, Sijtsma L (2017) Biorefinery of microalgal soluble proteins by sequential processing and membrane filtration. Bioresour Tech 225:151-158. https://doi.org/10.1016/j.biortech.2016.11.068

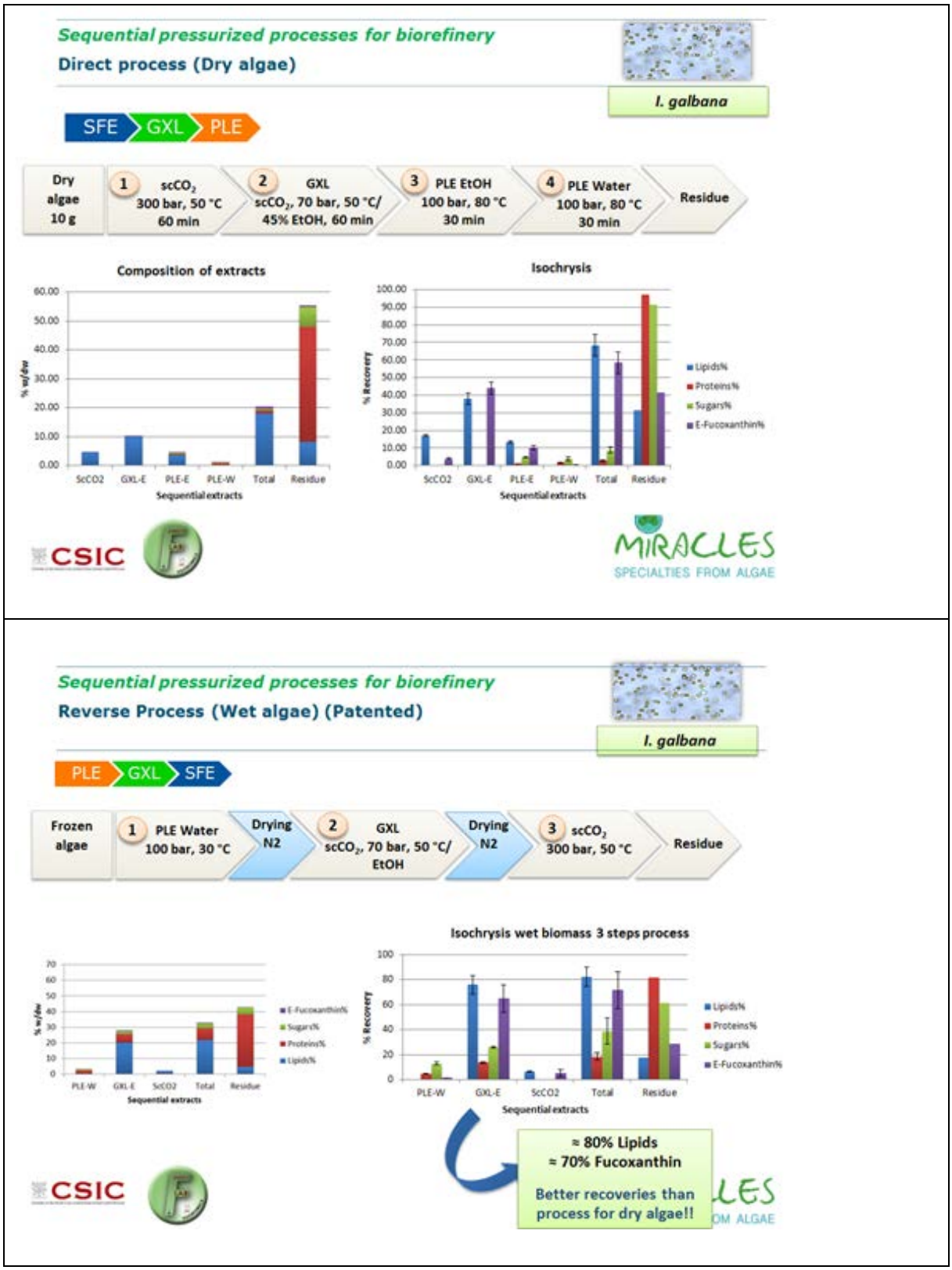

Figure 15 *TOP: Direct sequential extraction process to extract bioactive compounds from Isochrysis galbana, starting from dried biomass. BOTTOM: Reverse sequential extraction process using a reversed order of polarity extraction / solvents starting from wet biomass. With this new process, an increase of the pigment and lipid recoveries was obtained. The reverse process has been patented by CSIC.*

The extraction of polar lipids was also an aim. The third and fourth steps were performed with the objective of recovering fractions with high antioxidant activity, eventually rich in carbohydrates and proteins. The results obtained showed that the extraction process was partially selective according to the polarity of the solvent/mixture of solvents used. $ScCO_2$ extracts were rich in triacylglycerides and showed less carotenoid and chlorophyll contents than ethanolic extracts. The main carotenoid identified was fucoxanthin which was found in highest amount in GXL extracts obtained with 45% ethanol. Steps 3 and 4 provide extracts enriched in proteins and carbohydrates [11].

As a variant, a sequential extraction platform using a reversed order of polarity/extraction was developed with main aims of reducing costs and saving energy. This process started with the fresh, frozen biomass and consisted of three extraction steps: (1) PLE using water, (2) GXL using a mixture ScCO2:EtOH, (3) SFE using ScCO2 (***Figure 15 Bottom***). With this new process, a significant increase of the pigment and lipid recoveries was obtained while it also allowed a reduction of energy consuming steps such as freeze drying of the biomass. This reverse process has been patented by partner CSIC.

Furthermore, extraction and fractionation of bioactive compounds from the microalga *Scenedesmus obliquus* were performed. The process involved the following steps:(1) supercritical fluid extraction (SFE) using supercritical carbon dioxide (ScCO2); (2) gas expanded liquids (GXL) using 75% ethanol and 25% ScCO2 (v/v) and; (3) pressurized liquid extraction (PLE) using water. Extraction conditions were optimized using response surface methodology (RSM) and kinetic studies. Extraction yield, antioxidant activity as well as contents of total phenols, carotenoids, proteins and sugars were the studied response variables. High performance liquid chromatography coupled to evaporative light-scattering detector (HPLC-ELSD) analyses of the fractions revealed that triacylglycerols were mainly extracted by SFE. Lutein and β-carotene were the main pigments identified in the extracts by HPLC coupled to diode array and mass spectrometry detectors (HPLC-DAD-MS/MS), which were preferentially extracted in the GXL step. Lipids and lutein were recovered >70 %. Polar compounds such as proteins and sugars remained predominantly in the residue. Thus, the green downstream platform developed in this project for valorization of the microalgae biomass, is able to produce different fractions with potential application in the food, pharmaceutical and cosmetic industries[12].

Process integration and modelling

Five single product chains and three multiple product value chains were designed in SuperPro Designer® at industrial scale, based on the novel technologies/unit operations developed in MIRACLES complemented with (existing) benchmark technologies when required from the product specification. The results obtained at lab and pilot scale, were scaled up to a representative production scale of 10,000 tons y^{-1} of microalgal biomass. The three designed multiproduct biorefinery scenarios are:

1. Soluble proteins, pigments and peptides from *Nannochloropsis gaditana*;
2. Pigments and peptides from *Isochrysis galbana*;
3. Oils and peptides from *Nannochloropsis gaditana*;

[11] B. Gilbert-López, J. A. Mendiola, J. Fontecha, L. A. M. van den Broek, L. Sijtsma, A. Cifuentes, M. Herrero and E. Ibáñez, Green Chem., 2015, 17, 4599-4609.

[12] Gilbert-López B, Mendiola JA, Van den Broek LAM, Houweling-Tan B, Sijtsma L, Cifuentes A, Herrero M, Ibáñez E (2017) Green compressed fluid technologies for downstream processing of Scenedesmus obliquus in a biorefinery approach. Algal Res 24:111-121.

For each process the following results were obtained: 1) mass and energy balances 2) resources demand breakdown: utilities (energy and heating agents), materials, labour and consumables 3) size of the units. By combining these results a preliminary estimate and cost breakdown are given by the SuperPro model as total capital investment and unit processing cost (in Euro/kg biomass for CAPEX and OPEX) with a further breakdown in resources costs. With the mass and energy balances, also the water consumption and waste production can be obtained. The resulting data were used for 1) the market analysis (performed in WP4); 2) The scale up and demonstration work (WP5) and 3) The techno-economic and life cycle assessment (performed in WP6).

For all scenarios the following assumptions were made

- Solvents are always recycled as much as possible even if small losses (<5%) are included in the calculations of materials demand.
- The overall product yields are derived from combining mass balances and the initial biomass composition, which has been supplied by WP3 partners
- The overall energy consumption is derived from a combination of energy balances and utilities demand.
- Heat recovery is always implemented when the energy cascade allows it
- When possible the unit operations have been modelled as operating continuously. In case of batch operations (like for example the pressured liquid extraction) a detailed schedule has been implemented and scaled up to the investigated industrial scale.
- In case of multiple batch operations in sequence, their schedule has not been modelled separately, but integrated in a coherent and sequential way.
- Stressed biomass under N-starvation is considered for a lipid production value chain.

Based on these models, it can be concluded that the yield of biomass exploitation to marketable specialty products has increased from 7-30% for single product value chains to >95% for multiproduct value chains. The designed biorefiney scenarios were evaluated in detail via technoeconomic and envirionmental assessment, as reported in section 3.6 of this report.

3.4 Product development and market assessment (WP4)

Contributors: VFT (coordinator), EWOS/Cargill, SPAROS, BIOPOL, IMENZ, CHIMAR, NATAC, NOVA, ET, CE, URDV, DSM.

MIRACLES studied the incorporation of microalgae as whole cells or fractionated in different applications covering a broad variety of potential applications (***Figure 16)***

Figure 16. *Display of Miracles products during the conference Algae Biorefineries for Europe (17-18 Oct. 2017, Brussels, BE).*

Food applications

In the MIRACLES project promising results with respect to analysis and application of oil and protein derived from microalgae in food were obtained. Crude algae protein fractions were shown to have good emulsifying properties. Several emulsions were stable for at least several weeks with a spoonable, mayonnaise like consistency ***(Figure 17***).

Figure 17 *Preparation of protein emulsions and final products ('vegan mayonnaise').*

Detailed characterisation of the algal proteins responsible for emulsification is still lacking and therefore highly recommended, together with the determination of this specific protein yield on produced biomass. This is an essential requirement to study batch-to-batch variation during production and to enable future optimisation. Next to standardisation and optimisation of protein production, optimisation of downstream processing of the crude protein fractions (preferably using mild fractionation conditions) is required.

Lipid class analysis was performed on algae oil samples with focus on the Polar lipid fraction and TriAcylGlyceride fraction (both TAG content and Fatty acid profiles) and total lipid measurement was expressed in Total Fatty Acid. Promising is the fact that the obtained TAG fraction had a relatively high

~ 10 % EPA content. Total omega-3 LC PUFA of the crude algal oil was lower, compared to the omega-3 LC PUFA content of fish oil. A clear protocol for production of oil rich biomass production, which includes reproducibility, has to be established to avoid batch-to-batch variation. Further optimisation directed towards tailor-made edible oils is recommended, to produce algal oil which consists of both EPA and DHA at the fish oil level. And to underline the natural and sustainable character of these oils, in some food applications a light green colour and smell of algal oil might be attractive.

Therefore, systematic characterisation of the pleasant green smell and attractive green colour of some of the crude oil and protein fractions is highly recommenced as product stability of this green flavour and colour might open up novel applications of specific fractions from microalgae biorefinery activities. Finally, strain optimisation and process implementation towards combined oil (TAG) and protein production under normal, nutrient replete growth conditions would be recommended to enable cost effective production at the future supplier of food ingredients from microalgae.

Aquafeed applications

SPAROS Contribution

Aquaculture production has undergone remarkable growth during the past few decades, and it will continue to make a major contribution to meet higher demand for safe, healthy and convenient seafood products. Analysts expect aquaculture to provide an additional 23 million tons of seafood by 2030. Fish farming relies heavily on the use of compound feeds. The future growth of the animal feed industry faces great challenges, since traditional sources of high quality feed ingredients will not be able to meet the rising demands. Novel ingredients, serving as sustainable sources of protein and oils rich in long-chain polyunsaturated fatty acids (LC-PUFAs) are needed. Microalgae can serve these needs, since they show a high nutritional value, as a source of protein, omega-3 LC-PUFAs, such as EPA and DHA, vitamins, trace minerals, carotenoids and antioxidants.

Figure 18 *Manufacture of fish feed enriched with microalgae*

Several studies demonstrate that although dependent on species, microalgae can serve as sustainable raw materials in aquafeeds, contributing towards the lower use of marine-harvested resources, such as fishmeal and fish oil. Being an emergent industry, microalgae biomasses are not yet cost-competitive as a commodity feed ingredient.

In the framework of the MIRACLES project we have focused our research efforts towards the use of microalgae as a functional ingredient, in order to convey a benefit above and beyond the basic nutritional needs of fish. A series of feeding trials were conducted with gilthead seabream, the major farmed species in the Mediterranean region and Senegalese sole, an emergent high value species in Europe. Two microalgae, *Nannochloropsis gaditana* and *Phaeodactylum tricornutum* (as whole-cell biomass, broken-cell extracts and residual biomass and oil from biorefinery processes) were used in the diets of larvae, juvenile and market-size fish (See ***Figure 18***) to assess its effects on the overall growth performance, immune response towards stressful events and selected consumer quality traits. The project has demonstrated that a diet containing 5% of broken-cells extract of the fucoxanthin-rich microalgae *Phaeodactylum tricornutum* did not affect growth criteria, but significantly enhanced the survival of sole larvae. Moreover, larvae fed diets with microalgae showed a significant reduction of the skeletal malformation rate.

A diet containing 1% of *Phaeodactylum tricornutum* improved non-specific immune response to stressful events in sole juveniles. Seabream juveniles fed the diet with 1% broken-cells extract of *Phaeodactylum tricornutum* showed also a short-term beneficial response towards stress, with a clear stimulation of several immune-related pathways, measured by hematological and innate immune parameters and at the molecular level by a q-PCR array on liver and head-kidney tissues.

In adult seabream, the inclusion of 12.5% of *Nannochloropsis gaditana* defatted biomass and 3.64% of the extracted oil, resulting from a biorefinery approach, allowed the successful replacement of fishmeal and fish oil by 80% and 30%, respectively. In comparison to a current commercial formulation, the microalgae-rich diet did not affect the overall growth performance of fish and preserved a high nutritional value of seabream fillets in terms of omega-3 LC-PUFAS and health valuable minerals, such as selenium and iodine. Feeding seabream with the microalgae-rich diet was associated to a lower fat accumulation in the whole fish. Moreover, a test performed with a panel of 100 Portuguese consumers showed that seabream fed with the microalgae-rich diet scored higher than a current commercial feed, in terms of external appearance and brightness. Instrumental skin color measurements performed in the operculum area showed that fish fed the microalgae-rich diet had a higher lightness and a more vivid yellow pigmentation.

High standards of environmental sustainability, animal welfare and safety are among the top-ranked concerns of seafood consumers. Microalgae biomasses and products derived from an algae biorefinery approach show great potential as raw materials or functional ingredients in the aquafeed sector. Their use will contribute towards reducing the reliance on finite marine-harvested resources (replacement of fish meal and fish oil) and convey benefits beyond fulfilling the basic nutritional needs of fish in terms of animal welfare and product quality.

EWOS/Cargill contribution

Partner EWOS/Cargill performed digestibility and small salmon growth trials to indicate nutritional value of phototrophic EPA containing algae (*Phaeodactylum* and disrupted *Nannochloropsis*) for salmon (***Figure 19***).

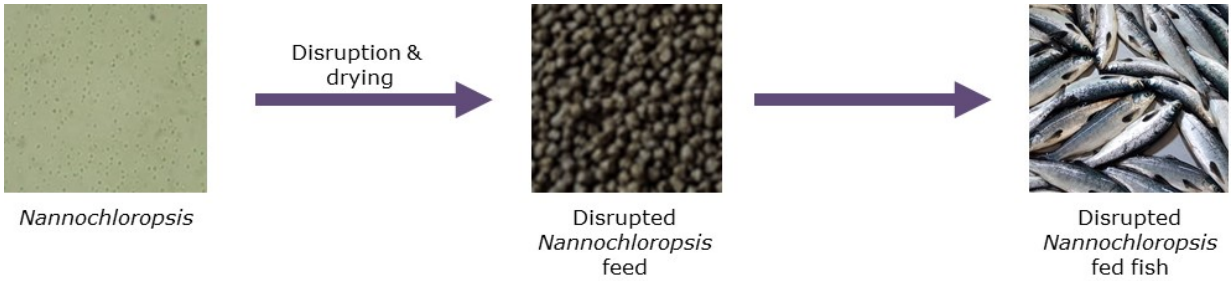

Figure 19. *Flowchart depicting Nannochloropsis algae to disrupted Nannochloropsis diet to disrupted Nannochloropsis fed fish in small freshwater salmon growth trial.*

The reduced digestibility observed for algae diets (especially disrupted *Nannochloropsis*) and generally low EPA+DHA% of product compared to other EPA+DHA sources (e.g., fish oil and heterotrophic algae) illustrate the potential challenge with using phototrophic algae meal as EPA/DHA source in salmon feed. However, growth performance of small freshwater salmon fed phototrophic EPA algae diets was not inferior to a reference diet. A natural antioxidant was added as extra protection for algae diets. However, the oxidation measures, peroxide value and TBARS[13], were not lower for algae diets compared to a reference despite extra natural antioxidant addition in algae diets and potentially harsh transport conditions of ingredients and diets.

Currently, there is no industrial application foreseen for phototrophic algae in salmon feed as EPA/DHA source due to relatively low volume production at high cost. However, the knowledge obtained on the nutritional value of phototrophic EPA algae in salmon feed can be utilised in further research with CO2Bio AS (algae pilot plant based in Norway; http://www.co2bio.no/en/) in which EWOS is a partner.

Cosmetic applications

Natac has validated the antioxidant bioactivity of the *Isochrysis galbana* extract rich in Fucoxanthin (2.6 %), obtained by the CSIC reverse sequential extraction process. Fucoxanthin is the best-known antioxidant found in *Isochrysis galbana*. However, the antioxidant activity of IGE-P complex has proved to be 100 times higher than that of pure fucoxanthin, suggesting the presence of other active compounds with antioxidant, stabilizing and/or synergistic properties in the extract.

[13] The TBARS (Thiobarbituric Acid Reactive Substances) assay is well-established for screening and monitoring lipid peroxidation.

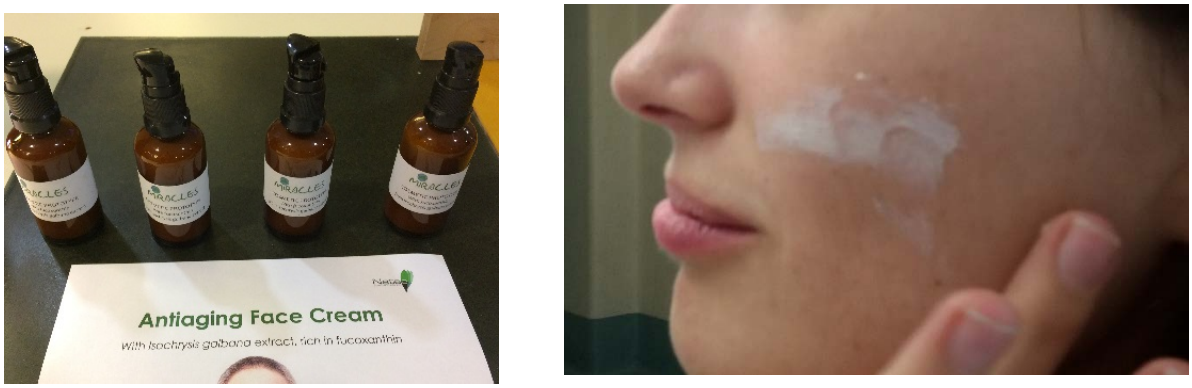

***Figure** 20 Face cream prototype and an example of skin application*

This outcome allows employing this ingredient in cosmetic applications, especially for dermo- cosmetic products. In order to enhance the stability of the extract, Natac has developed a phospholipid encapsulation process in order to obtain an innovative high added value ingredient for cosmetic applications (IGE-P complex). Additionally, phospholipids present certain physicochemical properties highly adequate for dermo-cosmetic applications. Natac has employed this ingredient to formulate two different prototypes - facial serum and facial cream – with potential antiaging and antioxidant properties In a final approach to the market, Natac has produced a pilot production of the facial cream (***Figure 20***) and has validated the stability of the bioactives (fucoxanthin and related carotenoids) present in the cosmetic.

Bioactive applications

In the MIRACLES project methods and procedures have been developed to produce bioactives from selected algal species. In particular, bioactive peptides were developed by partner Imenz that were shown to be 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. Furthermore Imenz demonstrated positive effects of algae derived fractions as a nutrient in industrial fermentation processes, which offers promising opportunities.

Bioplastics and biomaterials

In various experiments with different resin systems and microalgae types, the MIRACLES partners demonstrated the possibility to incorporate microalgae in thermoplastic compounds (Solanyl®; biodegradable starch-based resins made by Rodenburg Biopolymers suitable for injection moulding and in 2-component (thermo)setting systems (Touch of Nature® materials, made by Orineo[14]) (***Figure 21).***

[14] http://touchofnature.eu/

Figure 21 *Products made in MIRACLES using algae based materials. Left : bioplastic plant protection crate; Middle: bioplastic plant growing pot; Right: Example of Touch of Nature® thermosetting biomaterial, produced by Orineo.*

In both systems, microalgae are influencing the aesthetics of the end product. This is especially important with Touch of Nature® materials, as aesthetics are a Unique Selling Point of such materials. In thermoplastic systems, microalgae are increasing the stiffness, an interesting feature. But the most interesting property is the possibility to create a slow release system (Solanyl® is biodegradable), releasing microalgae slowly in the medium and delivering at a slow pace additional functionalities such as 'fertilising' the environment. Various applications were developed and will be further explored by the industrial partners.

Resin and glue applications

In the MIRACLES project partner CHIMAR evaluated microalgae biomass of various species, their protein fraction as well as residues after the extraction of lipids, in the synthesis of thermosetting polymers suitable to be used as adhesives in the production of plywood panels. It was found that microalgae-based biomass can successfully replace a 30% of phenol in phenol-formaldehyde resins and the plywood panels produced with them may be used at both interior and exterior applications according to EU standards. Furthermore, microalgae biomass was successfully used for the development of bio-based finishing material suitable for wood-based panels (***Figure 22)***.

Figure 22*. Plywood panels and coating material produced using algae based resins*

Fertiliser applications

The strategy within the MIRACLES project concerning agricultural valorisation has been putting the focus on added value with a high-end perspective well beyond a normal fertilising effect. Using "residues" of the biorefinery of algae as an (additional) fertilizer had a low priority. This strategy appeared to be the right one. Three preparations are selected based on extensive experimental work and ample practice validations (***Figure 23***)

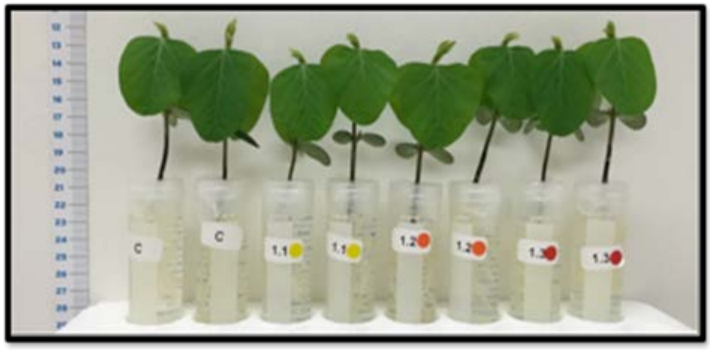

Figure 23 *Set up of bioassays*

1. An *Isochrysis* preparation has been proven to counteract stress imposed on plants by drought and low light conditions. The same preparation enlarges the resistance of ornamental pot plants to stress in the logistic phase
2. A *Nannochloropsis* preparation showed capacity to induce the formation of flower buds, meaning for example that initially vegetative buds are transformed to reproductive ones. The same preparation showed anti-stress action to chilling and cold stresses in preliminary experiments
3. A *Pheaodactylum* preparation induced an increased nitrate-use-efficiency in wheat and maize seedlings by a double action; bringing crop production at lower levels of fertilizer a step further:
    a. The uptake system for nitrate develops a higher affinity for nitrate, meaning the plant growth can continue under minimal nitrate contents in the root environment
    b. The first step in the biochemical pathway, nitrate reductase system, bringing nitrate to amino acids showed an increased capacity.

Further development and commercialisation of these findings is ongoing.

3.5 Demonstration of integrated value chains (WP5)

Main contributors: Fitoplancton (Coordinator), WFBR/DLO, CSIC, EWOS/Cargill, SPAROS, BIOPOL, CHIMAR, NATAC, ET, CE, URDV.

The main objective was to demonstrate selected integrated value chains to deliver proof-of-concept and demonstrate techno-economic viability. Specific aims were:

- Pilot scale production of algae batches with optimized composition;
- Validation of selected processes and application testing at pilot scale;
- Selection of value chains for demonstration;
- To demonstrate feasibility of selected integrated process chains for different target products.

All demonstration activities were focused on the actual biorefinery process chains, that is, starting from the algae biomass, followed by biorefinery and validation of product application. All lessons learned during the project were integrated in these activities.

The selection of value chains for demonstration was based on defined industrial criteria (most important one first) : 1) technological readiness for scale-up of the process; 2) feasibility of the process according to the techno-economic assessment 3) impact and market awareness; 4) availability of similar products in the market ; 5) multiproduct value chains + co-products: 6) strain diversity, a single one or several involved in the different processes; 7) the availability of realistic biorefinery scenarios. According to these criteria, validation took place at either pilot or laboratory scale. For each chain a protocol was developed, and product samples for dissemination and exploitation were made available by the involved partners.

Five product chains were valdated on pilot scale.

- **Production of oil-enriched algal biomass and solvent extraction of oil for food and feed.**

Fitoplancton performed pilot trials with nitrogen starved *Nannochloropsis* cultivation for enhanced oil production enriched in TriAcylGlycerides (TAG). Data demonstrated that oil enrichment of microalgae biomass under nitrogen starvation is feasible under strict control of cultivation parameters, in particular temperature**.** Following disruption of the algae, EcoTreasures performed pilot scale oil extraction following the protocol shown in ***Figure 24***. Both the oil and the defatted algae biomass were tested by Sparos in fish feeding trials with promising results. Furthermore the oil was analysed by URDV to assess its suitability for use in food products.,

- **Manufacture of microalgae-enriched aquafeeds.** An adapted protocol was developed to include algae products (dry microalgae, microalgae-derived oil, defatted biomass) in aquafeed diets, shown in ***Figure 25***. Feeding trials were conducted by partners Sparos and EWOS to assess the produced algae enriched feeds.

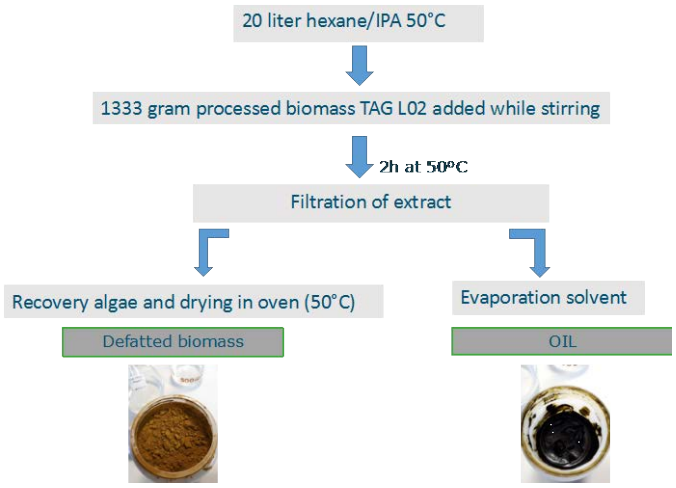

Figure 24. *Flowchart depicting the production of a crude oil and defatted biomass from microalgae.*

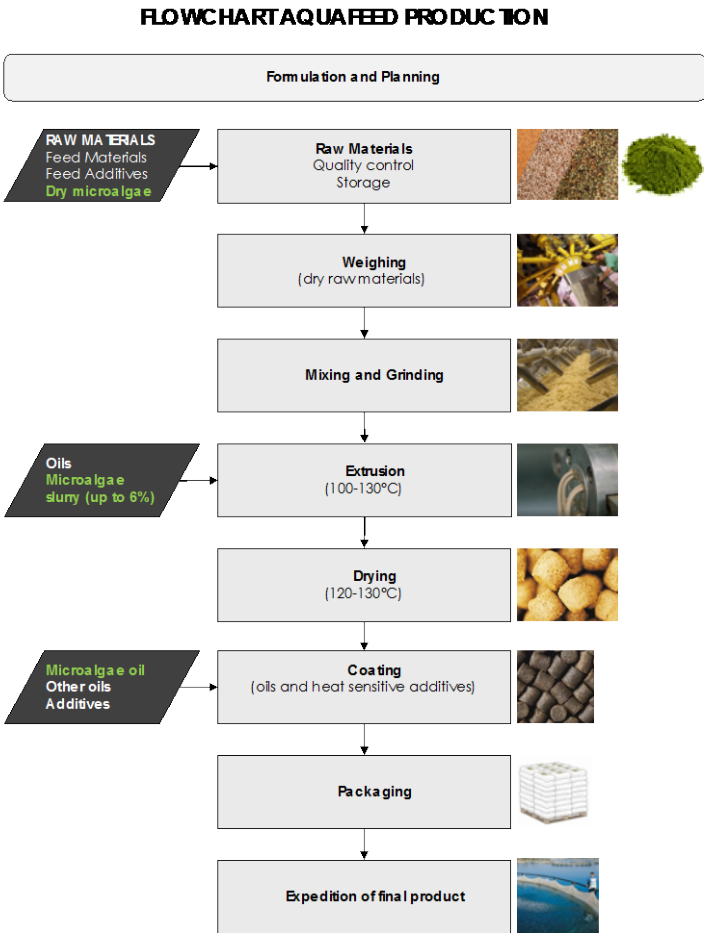

Figure 25. *Flowchart depicting the process for the elaboration of aquafeed with microalgae*

- **Synthesis of microalgae-based resins and manufacturing of plywood panels.**

Several algae species and processed fractions were successfully employed as phenol substitute in the synthesis of resins, rendering panels suitable for interior and exterior conditions according to EU standards (**Figure 26**). The developed technologies, although proven at pilot scale, require further validation and fine tuning on larger scale prior to their final industrial application.

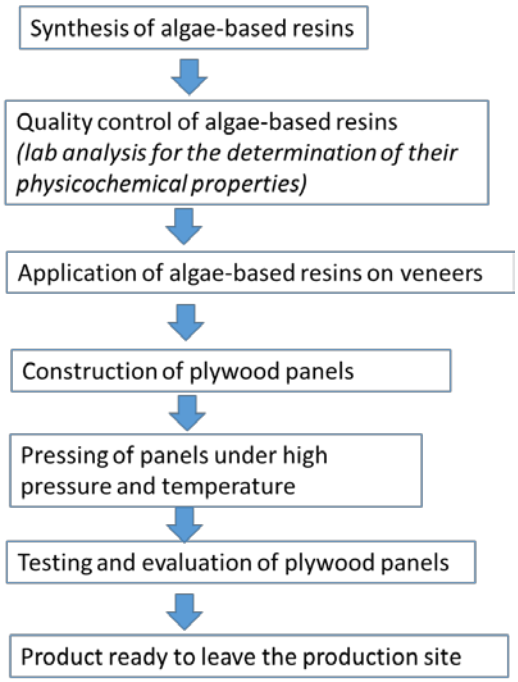

Figure 26. *Overview of process for the elaboration of wood-based panels using microalgae.*

- **Production of thermoplastics containing microalgae for horticulture and other applications.**

Different resin systems and algae materials were used to demonstrate production of a Solanyl®-algae compound including use in various injection moulding applications.

- **Production of microalgae-based thermosetting aesthetic biomaterials.**

Excellent performance of microalgae materials was demonstrated in translucent sheets and as aesthetic filler in thermosetting biomaterials (Touch of nature®). Specifically developed protocols preserved the colour of the algae, an advantageous feature in the final products.

Two production chains were validated on smaller scale i.e:

- **Production and use of soluble microalgae protein fractions in food applications.**

The process complexity of protein purification and the limited yields were considered in order to decide on the laboratory-scale of the assay. Model food emulsions ("vegan mayonnaise") were successfully elaborated from crude microalgae protein fractions (see 3.4) . Final results were successful in terms of rheological and organoleptic properties of the products with good emulsifying properties and stability. An adapted protocol was developed.

- **Production of a fucoxanthin-enriched extract and formulation of cosmetics**

The proof of concept, including formulation and pilot production of facial cream prototypes was successfully delivered by NATAC. A full protocol was developed for microalgae extraction and elaboration of the cosmetic product.

Overall, the results of the demonstration and validation activities in the framework of MIRACLES provide a strong basis for further development and scale-up.

3.6 Techno-economic assessment and life cycle assessment integrated value chains (WP6)

Contributors: NOVA (Coordinator), WU, VFT with input by all partners.

Technoeconomic assessment

The project results were integrated in value chain scenarios and evaluated via techno-economic analysis, Life Cycle Assessment and socio-economic assessment. The main technological developments from the project in cultivation, harvesting and medium recycling and the developed biorefinery flowsheets and product applications were integrated in these scenarios. Data were provided by the partners, complemented where needed with best practice data from partners and other sources.

The biorefinery scenarios studied in MIRACLES can be split in 2 categories:

- Five single product scenarios (SP): one main product to be extracted and a large remaining residue with limited market value;
- Three Multiproduct scenarios (MP): various value products obtained from algae biomass along with residue streams that are as much as possible valorized as co-products.

As key assumption, the refinery schemes were aligned and scaled to the cultivation and processing of 10,000 t dry algae biomass per year. Current global microalgae production is ca. 40,000 ton dry weight, of which > 500 ton in Europe[15]. To evaluate algae as a potential crop of the future[16] their potential at larger scale needs to be appraised to highlight the opportunities and challenges. This justifies the 10,000 ton scale, which corresponds to a cultivation surface area (Southern Spain) of 500-840 ha for biomass grown under optimum (ca. 20 ton/ha.yr) and nutrient limited conditons for enhancement of oil production (ca. 12 ton/ha.yr) respectively. This area can be compared to the greenhouse horticulture sector in the Netherlands with a total area of 9,300 ha in 2016. [17]

Elements and assumptions in the designed chains are:

- CO_2 supply via fixed bed adsorption from air using technology and data developed by UT in WP1. The required CO_2 to produce 10.000 ton biomass, sums up to 20.000 ton CO_2 per year. This is bound from $4.4*10^{10}$ m^3 of air using $5.4*10^4$ kg sorbent at a cost of ca. € 0.16/kg CO_2.
- Algae cultivation in the South of Spain in a novel photobioreactor consisting of PolyEthylene (PE) bags, to a final algae concentration of 2 g/L. The minerals and water consumption were taken into account, as well as the electricity for operating the systems. The cultivation cost for the given production conditions (Spain, 500-840 ha) are €4,50/kg algae (normal growth conditions) and €7,50/kg algae (stressed growth conditions under nutrient limitation to enhance oil production)[18] Data were provided by algae producer Fitoplancton.

[15] Value and Size of the Algae Biomass sector in Europe in 2016: current relevance and future potential for Biorefinery. Vítor Verdelho Vieira, Algaebiorefineries for Europe, 2017.
[16] Microalgae: Crops for the future, Philippe Willems, Algaebiorefineries for Europe, 2017.
[17] http://www.agrimatie.nl/SectorResultaat.aspx?subpubID=2290&sectorID=2240&themaID=2286&indicatorID=2014
[18] In specific algae strains nitrogen limitation leads to enhanced oil accumulation while at the same time reducing overall biomass productivity. An optimum balance must be found. The higher cost for lipid-rich biomass is due to the extended cultivation period and higher use of utilities and materials.

- The algae are harvested continuously using the submerged membranes as developed by VITO (WP1) and concentrates the algae suspension 10-fold. The membrane filtration allows recycling of the medium to the cultivation system; 75% medium recycling was assumed.
- Centrifugation is applied for further concentration of the algae suspension. Simulations have shown that a combination of 10-fold concentration using membrane filtration followed by further concentration using centrifuges is the most economical combination. As a result the electricity consumption is relatively low and the recovery is 98%.
- The design and cost estimate of processing/biorefinery of the biomass was based on data and flowsheets developed in the project in WP3.
- For each scenario the production costs and potential revenues were evaluated by Nova and VFT based on representative data for market value of products from a database developed by VFT with input by the industrial partners

Single product scenarios

The MIRACLES project evaluated 5 different single product scenarios (SP):

- Whole dried cells that can be sold in aquafeed, in materials, as food ingredient, etc.
- Disrupted cells (=broken cell walls) to improve digestibility in a.o. aquafeed
- Soluble protein used as emulsifiers or gelling agents in food, cosmetics, adhesives, etc.
- Pigments as colorants and anti-oxidants for food, cosmetics and materials
- Omega-3 oil source for food and aquafeed

The processes associated with each scenario are depicted in ***Figure 27***.

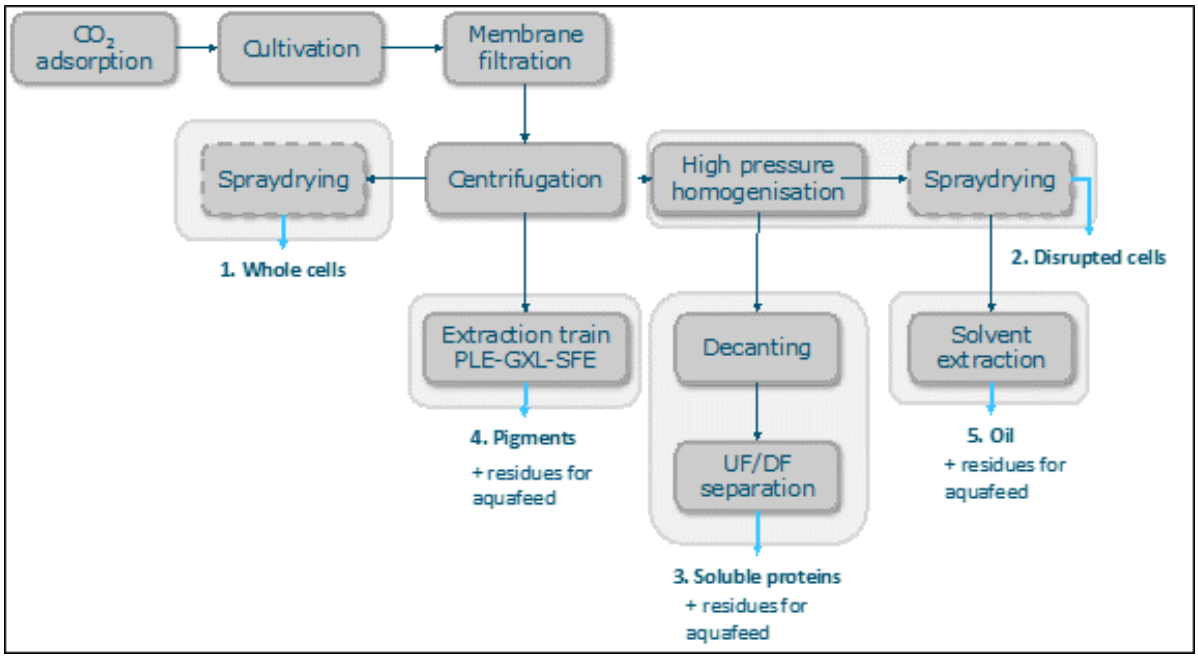

Figure 27*. Single product scenarios*

The economic evaluation shows that the biomass costs for all single product scenarios exceed the biomass value and these chains are therefore not economically feasible. This is due to the low market value of the considered applications for whole or broken cells and the limited fraction of the main valuable component in the biomass combined with a limited value of the (large) residue streams.

Multiproduct scenarios

In order to extract more value from the algae biomass, mainly the residues, 3 multiproduct scenarios (MP) were considered. These multi-product concepts aim at full utilization of the biomass and are promising options as they 1) improve overall yields and the yield of marketable (co)products, 2) reduce waste, and 3) reduce the environmental impact per component.

MP1: Proteins, pigments, peptides

The first multiproduct chain (***Figure 28***) uses *Nannochloropsis gaditana* and leads to 5 (co)products and decoloured spent biomass. First, soluble proteins are obtained. Also, a polysaccharide rich stream and a stream with sugars and salt are gained in the ultrafiltration step. The solids from the decanting step are dried after which a 2-step extraction process is applied. This results in a pigment-rich extract and a protein/sugar extract. The remaining biomass is subjected to alkaline conditions to solubilise the proteins, which are separated from the decoloured spent biomass using centrifugation (peptide extraction). The solution with proteins is hydrolysed using enzymes to obtain peptides. This scenario yields 3 main products: soluble proteins, pigments and peptides; 3 side-streams: lipids, polysaccharides and decoloured pellets, brought together in 1 residue/side stream.

MP1: proteins, pigments, peptides

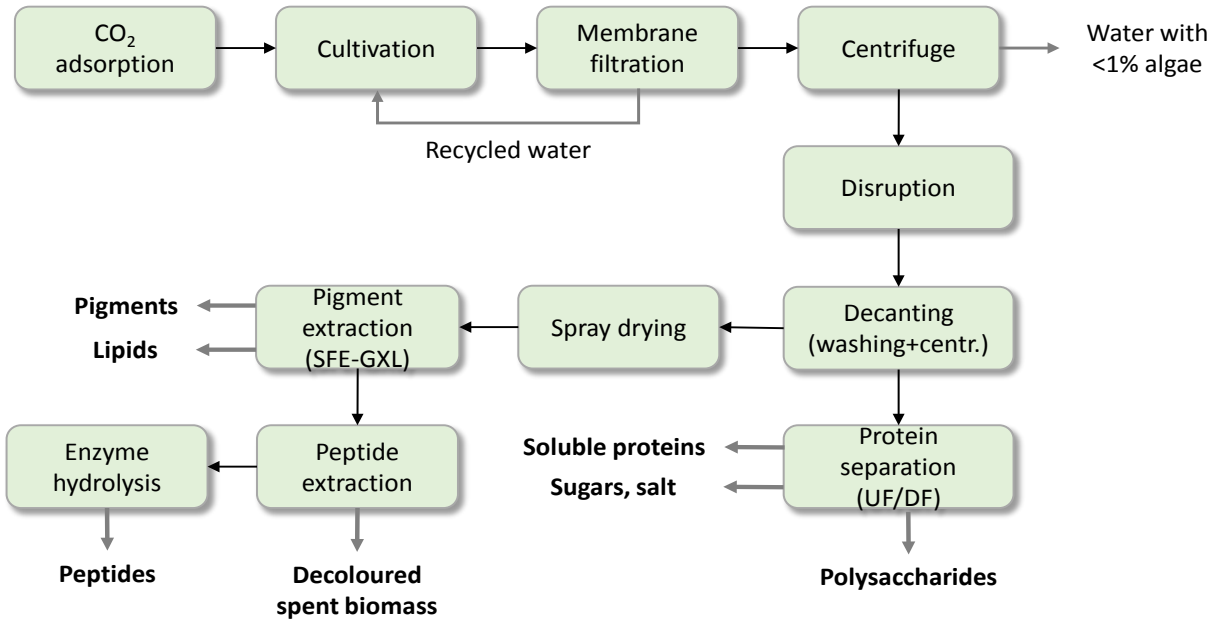

Figure 28. *Multi product scenario MP1 protein-pigment-peptides*

Potential applications:

- Soluble proteins as emulsifier in food and cosmetics or in adhesives
- Pigments as colorant or anti-oxidant in food and cosmetic
- Peptides as preservative in food and cosmetics; as flavour enhancer; as fermentation additive
- Residue as aquafeed

Cost structure:

- CAPEX: €220 million

- OPEX: €5.400/T
- Yield: 24% pigment extract; 18% peptides, 7% proteins, 34% residue (to aquafeed)
- Total cost: €8.000/T biomass
- Biomass value: €10.900/T biomass

MP2: Pigments, peptides

The second multiproduct chain uses *Isochrysis galbana* and results in 3 (co)products (***Figure 29***). The first process step is a 3-step extraction train. The first main product is a pigment-rich extract with antioxidant properties. Simultaneously a lipid extract is obtained, however this is only 0.3% of the starting biomass. In addition, a protein/sugar rich extract is produced. This extract is combined with the decoloured biomass. This mix is used in the alkaline extraction to solubilise the proteins, which are hydrolysed into peptides. The MP2 scenario yields two main products, pigment extract and peptides and 2 residues: one containing some residual proteins, carbohydrates and salts; the other rich in lipids.

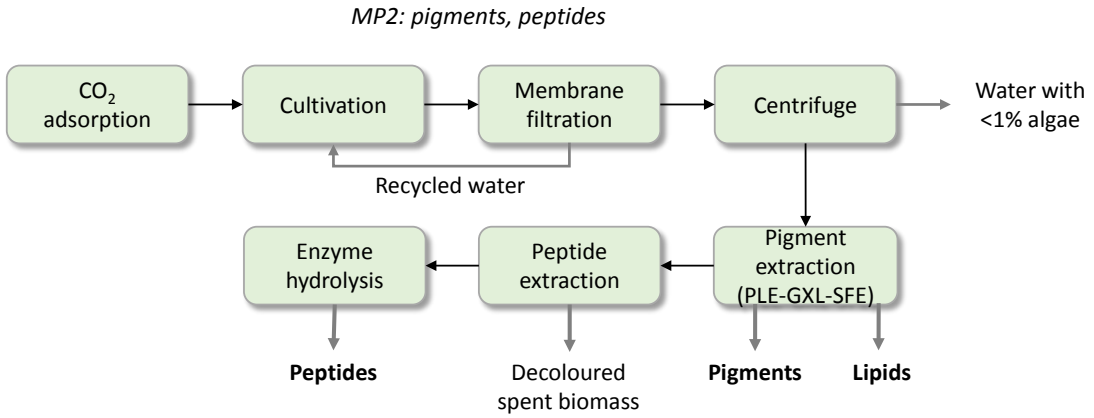

Figure 29. *Multi product scenario MP2 pigment-peptides*

Potential applications:

- Pigments as colorant or anti-oxidant in food and cosmetics
- Peptides as preservative in food and cosmetics; as flavour enhancer; as fermentation additive
- Residue 1 as fertiliser
- Residue 2 as lipid supplement for aquafeed

Cost structure:

- CAPEX: €130 million
- OPEX: €5.800/T
- Yield: 27% pigment extract; 54% peptides; 15% residue 1; 3% lipid residue
- Total cost: €7.100/T biomass
- Biomass value: €12.000/T biomass.

MP3: Oil, peptides

The third multiproduct chain uses stressed *Nannochloropsis gaditana* cultivated under nitrogen limitation with elevated lipid content ***(Figure 30).*** After drying and cell disruption the oil is extracted using a mixture of hexane and isopropanol. The de-oiled biomass is used in the alkaline extraction to solubilise the proteins, which are enzymatically hydrolysed into peptides. This scenario yields two main

products, oil and peptides and 2 residues: a coloured residue containing some residual proteins, carbohydrates and salts and a lipid residue (after omega-3 concentration).

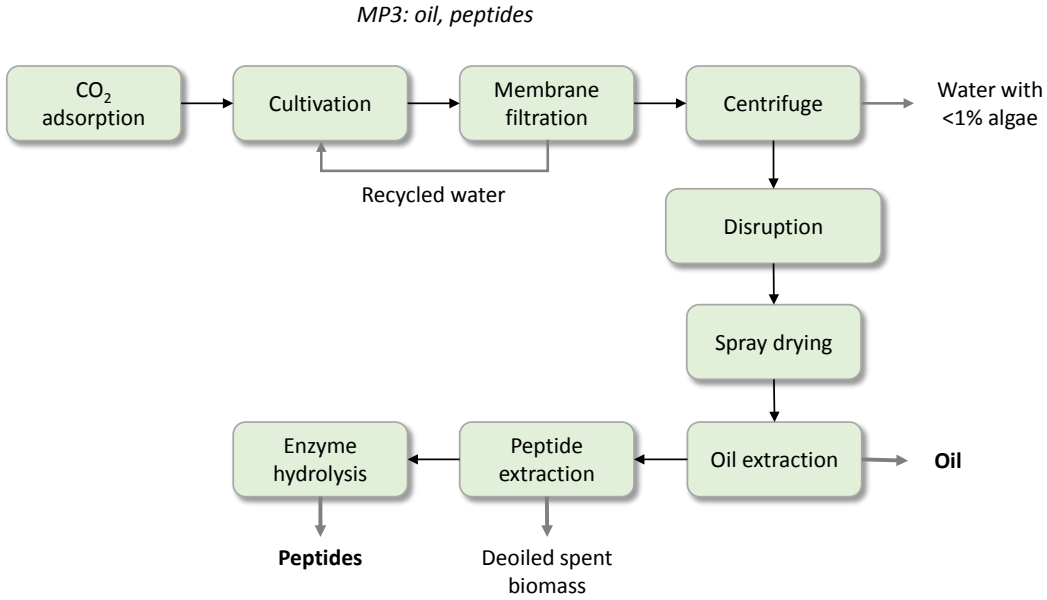

Figure 30: *Outline of the multiproduct chain MP3 for oil and peptides production.*

Potential applications:

- Oils enriched in EPA omega-3 in food (spreads, sauces, dressings, etc.) and aquafeed (salmon)
- Peptides as preservative in food and cosmetics; as flavour enhancer; as fermentation additive
- Coloured residue (deoiled pellet) as plant growth promoter, aesthetic additive for thermoplastics and thermosets
- Lipid residue (after omega-3 concentration) as lipid supplement for aquafeed

Cost structure:

- CAPEX: €90 million
- OPEX: €7.900/T
- Yield: 30% peptides, 25% EPA65 oil, 30% coloured residue, 15% lipid residue
- Total cost: €8.800/T
- Biomass value: €10.300/T biomass

The total production cost of the multiproduct chains (incl. biomass production and biorefinery) is between € 7.10 and € 8.80 per kg algae.

The results indicate that microalgae cultivation and harvesting contribute 60-70% to the total production costs (MP1, MP2) to 85% when producing oil and peptides from stressed biomass (MP3). The biorefinery costs are lowest for MP3 (oil, peptides) and increase for MP2 (pigments, peptides) and MP1 (proteins, pigments, peptides).

The potential profitability of the value chains was evaluated. The results show that the three multiproduct scenarios have a much better potential profitability compared to the single product scenarios. Additional insights in product functionality, quality and market size are needed to narrow down the range of foreseen product revenues.

The trends in potential profitability were further evaluated and confirmed by the performance of a financial analysis by VFT in the business plan according to the rules of the art. First the IRR (Internal Rate of Return) of the base cases was calculated, followed by a simulation to reach an attractive IRR=20% (= the interest rate an investor can expect) by varying the production cost and the product value. This gave most interesting results. Single product scenarios are still far from profitability, with production cost higher than product value. Multiproduct scenarios however show an interesting potential, with base case IRR from 8 to 18%: the 20% target within reach.

Life Cycle Assessment integrated value chains (WP6)

The technical evaluation revealed that cultivation is the dominant water consumption process, even with extensive medium recycling. Also regarding electricity consumption, cultivation is the main contributor for all value chains. The electricity consumption varies between the multi-product value chains from 20 – 32 kWh/kg algae, heat consumption (for processing) varies from 4 – 17 kWh/kg algae.

A screening Life Cycle Assessment (LCA) was performed to quantify major environmental impacts in the three multiproduct value chains, including the contribution to Global Warming, Acidification, Eutrophication, Photochemical Ozone Formation and Abiotic Depletion (fossil fuels). A hotspot analysis was performed to identify improvement strategies for future optimization.

The LCA results clearly show that cultivation in photobioreactors is a hotspot from an 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 CO_2), heat for drying and electricity consumption. (***Figure 31***). Further improvements in solvent recycling can 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.

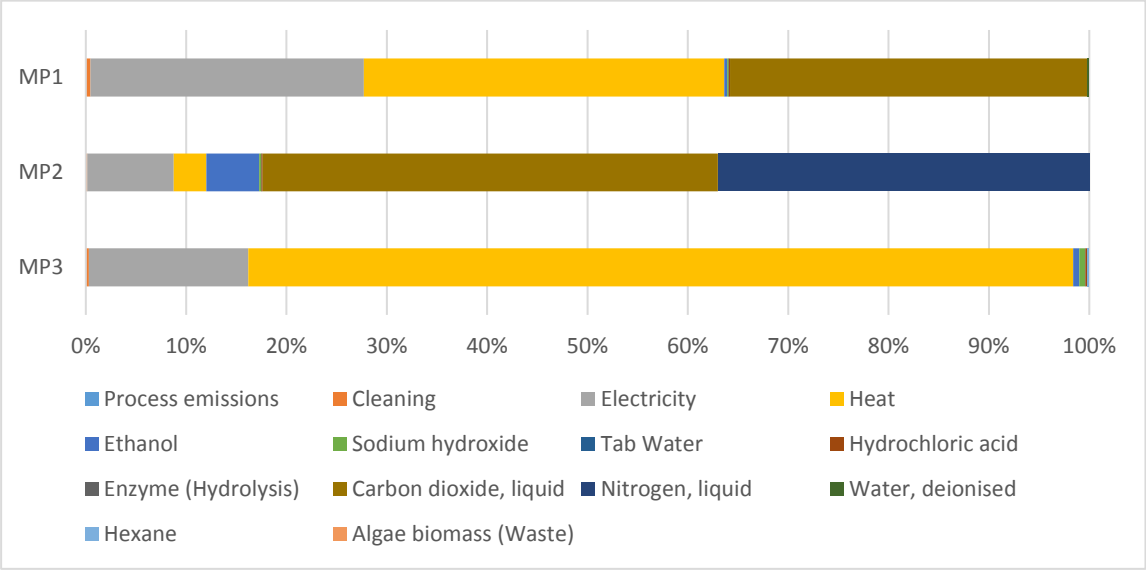

***Figure 31**: Hot spots of the three multi-product biorefinery scenarios (excluding cultivation)*

On a product level, algae biomass derived products are not yet environmentally competitive with alternatives[19] 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 and the early development stage of the used extraction techniques. Further improvements like a shift to renewable energy and other improvements are necessary to make algae a sustainable solution. These results are not surprising since agricultural practices and processing have been optimised extensively. Moreover, conventional sources of protein and oils have specific issues and concerns which are not or only partly covered in a Life Cycle Assessment including socio-economic and biodiversity impacts involving deforestation for palm oil and soybeans, overfishing for fish oil, and land use in general. Further conclusions and recommendations from the LCA are:

- Medium recycling substantially lowers the net water use as well as nutrient consumption and should always be included in the design of an algae system.
- Production of algae under nutrient limited conditions to enhance the oil content is a critical issue seeing the reduction in (overall) production efficiency. The merits of this strategy should be carefully evaluated in terms of added value and environmental impact.
- Methodological choices e.g. with regard to allocation have a big influence on results. This should be taken into consideration, when interpreting results. Especially when comparing algae proteins to alternative, conventional protein sources, allocation should be done at least on economic basis as well as on mass basis for transparency.
- This study performed an attributional LCA based on mostly lab scale data complemented with literature data and some extrapolations to commercial scale. A prospective LCA which includes a scale-up of the system as well as the full implementation of the improvement measures is recommended, to evaluate the full potential of micro-algae and to be economically and ecologically more competitive and sustainable.

[19] The environmental impact of algal protein was compared to conventional protein sources: soybeans, rape seed, skimmed milk, DDGS and fishmeal. Algae oil was compared with palm oil, soy bean oil, rape seed oil and fish oil from Peru and Norway.

3.7 Conclusions and next steps

The MIRACLES consortium has successfully developed technological innovations in algae production, harvesting and processing, improved biorefinery technologies and new, validated specialty products for application in food, aquaculture and non-food.
The project has achieved substantial progress beyond the state of the art and a large number of exploitable results for further development and commercialization. This includes technologies, new product applications and business models, supported by a marketing and business plan and extensive dissemination and exploitation activities.

The developed technologies are now at a TRL level of 4 and 5, with exceptions in both directions. Further research and demonstration are required to validate technologies and products at TRL 7-9 and to refine the business cases. Also the ideal plant size according to the selected product mix needs to be established and detailed modelling performed to refine the business cases.

The profitable business potential of the designed multiproduct biorefinery scenarios shows that this approach should be further developed and applied to other algae strains and products in order to promote further development of algae specialties. Ultimately, specialties can bridge the gap towards commodities from algae.

Based on the results of the techno-economic and environmental assessment, improvements that will increase profitability and reduce the LCA impacts were identified. Further R&D on these topics is recommended with focus on algae production (as the main cost factor) and biorefinery processing i.e.:

- Reduction of cultivation cost and enhancement of productivity and yield of target products via strain improvement, operational adapatations and system improvements;
- Energy saving measures and use of on-site generated renewable energy;
- Optimization of processing to achieve enhanced product recovery, reduction of solvents use:
    - Enhancing yields of soluble protein extraction;
    - Reducing capital expenses of pigment extraction;
    - Optimising oil extraction using biomass grown under optimal (non-nutrient limited) conditions, using green solvents;
- Process integration of the biorefinery by coupling condensation, evaporation , heating and cooling steps.

4 Potential impact

4.1 Socioeconomic impact

Microalgae can be grown on land unsuitable for agriculture, use seawater instead of valuable fresh water resources and are a source of unique products. For successful commercialization, technologies need to be optimised and scaled up; business models need to be demonstrated. The results from MIRACLES contribute to this goal by successful development and demonstration of

- 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

Through cost reduction and value creation the results contribute to widen the gap between production costs and market value of algae specialties, demonstrating commercial feasibility and potential profitability of a microalgae venture.

COST REDUCTION:
Technological innovations on the level of CO_2 concentration, algae production, algae harvesting and extraction processes with high cost reduction potential.

VALUE CREATION:
Demonstration of high profit applications in food, feed and industrial markets supported by an innovative positioning strategy.

The developed multiproduct scenarios in which various, marketable added value products are obtained show a profitable business potential. This is caused by new cost reduction technologies developed within MIRACLES, combined with value creating new application developments and an innovative positioning strategy.

The final results of the project thus 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 make this sector more attractive to investments with a positive impact on employment.

The ‘blue' economy in the EU as a whole represents 5.4 million jobs and a gross added value of ca. € 500 billion per year. Development in this area is promoted by the EU’s long-term *Blue Growth strategy* to support sustainable growth in the marine and maritime sectors. MIRACLES contributes to this strategy via development of sustainable jobs and growth in microalgae biotechnology and the aquaculture sector. Furthermore results from MIRACLES contribute to several of the EU’s economic (competitiveness), societal (prosperity, knowledge based society) and environmental policies and priorities, including:

- *Resource-efficient Europe*: MIRACLES results will "boost economic performance while reducing resource use" by using algae as a source of new products some of which have the potential to substitute fossil resources (saving CO_2) or natural resources that are currently overexploited;
- *Agenda for new skills & jobs*, promoting research in Life Sciences, marine science, bio-based chemicals and bioprocessing as well as entrepreneurial potential, making it attractive for young researchers;
- *Innovating for Sustainable Growth - a Bioeconomy for Europe.* This EC Strategy aims to promote the development of a biobased economy with special emphasis on the role of SMEs.

Role of the consumers

To reflect societal impact "Consumers" are a very interesting and powerful stakeholder group, while only limited knowledge about their attitude towards algae is available. Therefore, a screening consumer study was performed by URDV, Nova and VFT 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 assessment was segmented in three surveys, one for each product category food, aquafeed and cosmetics. The number of respondents sums up to 156 for the food survey, and 71 each for the aqua feed and cosmetics survey. Statements were used to indicate whether the respondent "agrees" or "disagrees" with the provided arguments, in addition to open questions. The study provides first insights of perceived benefits and concerns and can serve as a basis for further in-depth investigations.

The **results of the food survey** (Figure 32) indicate that algae have in general a positive image concerning nutrition and sustainability as they are vegetarian and have the image of being nutritious and environment friendly (land use and local production). Microalgae are also recognized as 'new food' and 'fun food'. For application in food potential off-taste and smell are a major concern. Further concerns include potential toxin production and (heavy-metal) contamination. Certification might be a means to communicate a guarantee that algae are not harmful to consumers.

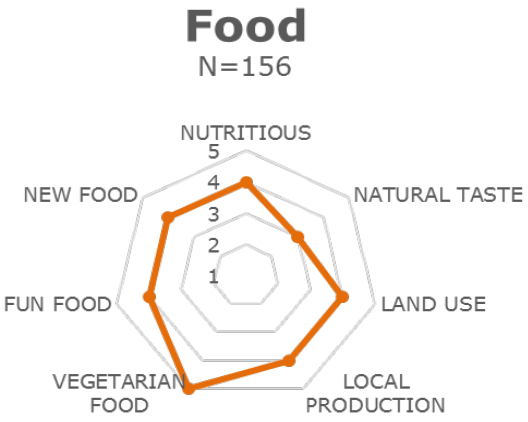

Figure 32 *Evaluation of the food survey. Scale : 1 is „I strongly disagree" and 5 is „I strongly agree".*

In the case of aqua feed ***(Figure 33***) sustainability aspects (e.g. land preservation, wildlife protection) of algae-based fish feed is a major argument of potential consumers. Functional arguments such as the influence of microalgae on the appearance of the fish skin , the meat texture, colour and taste are less convincing, however, we cannot say if these arguments are in general less important or if respondents

(mainly from North-Western Europe) lack the experience. Added arguments mainly include that low-cost algae feed may reduce fish price and increase the availability of fish. Like for food, contamination is a concern and a scientific proof whether algae-fed fish contains more or less contaminants could support the communication about algae-fed fish.

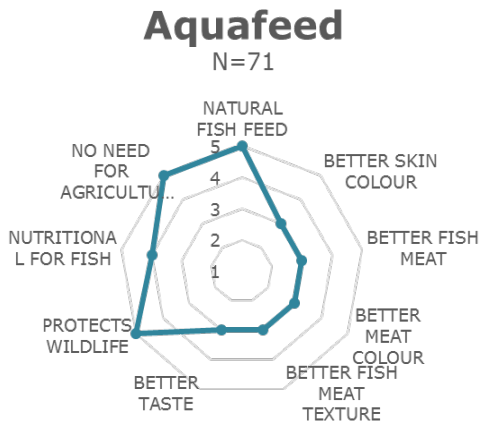

***Figure 33**. Evaluation aquafeed survey. Scale: 1 is „I strongly disagree" and 5 is „I strongly agree".*

In the case of cosmetics *(Figure 34*) we see a general positive attitude: respondents clearly appreciate the use of microalgae in cosmetics. The overall responses regarding algae-based cosmetics were generally positive and quite homogenous throughout gender, professionals vs. consumers and innovativeness. Risk of toxins and allergens are relatively minor concerns. Natural ingredient, versatile ingredient (multifunctional) giving a natural colour to cosmetics, environmental-friendly ingredient are all strong arguments. And using the anti-microbial properties of some algae peptides as preservative, along with other properties is a good marketing strategy.

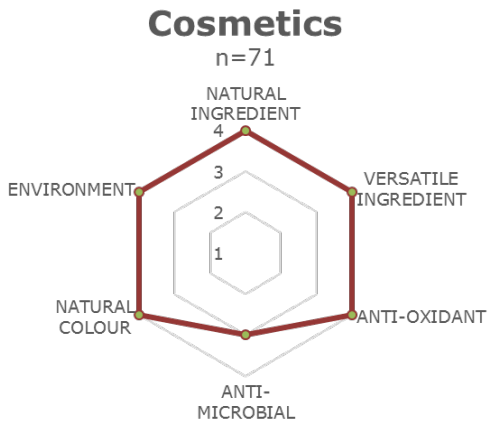

***Figure 34**. Evaluation cosmetics survey. Scale: 1 is „I strongly disagree" and 4 is „I strongly agree".*

The results of the consumer study provide useful first insights into the consumer perspective of algae-based products, and indicates clear trends. In general, the environmental arguments associated with microalgae (no use of arable land, wildlife protection, local production,…) and the health aspects of algae-based food are well perceived. Overall, consumers seem to be open minded and interested in

algae products. Concerns are mainly related to off-taste, off-smell and purity (toxins, contaminants). These concerns are most interesting when positioning and communicating about microalgae in the market. All actions and precautions need to be taken to reassure the consumers that their concerns are well addressed via appropriate actions incl. quality control in production, processing and formulation and proper communication.

Comparing LCA data of algae products with conventional agricultural products (e.g. soluble proteins and oil) still turns out negative. This is due to the high energy consumption in algae cultivation and processing as well as the early development stage of the used extraction techniques. Further improvements and upscaling are required to make algae a sustainable solution.

Regarding socio-economic impacts, however, algae have significant advantages compared to alternative plant and animal resources. Especially conventional oil and protein resources have associated problem fields, e.g. overfishing for fish meal, deforestation and habitat loss in the case of soy and palm oil plantations and negative socio-economic impacts on health issues and (in)-direct effects of land-use. Algae can contribute to at least a partial alleviation of these societal concerns.
Local algae cultivation can provide employment and income. However, it should also be noted, that in some areas, agricultural expansion is considered to contribute to economic growth and provides jobs and income for local people. Whether or not algae contribute to the same extent depends on many factors that cannot be answered on a general level, but only in a specific context with defined biorefinery products in a specific location. On the other hand expansion of agriculture may have negative consequences, such as displacement of local farmers to marginal lands and lack of participation in the use of local resources such as land and water. Whether the one or the other is true depends highly on the resource and area where it is extracted as well as the governance in the region. It is strongly recommended that algae are cultivated on non-arable land to avoid land use change and the competition with food crops. Furthermore local conditions and socio-economic benefits of current practice should be carefully addressed when implementing algae cultivation.

Overall, MIRACLES made valuable contributions to further development, industrial implementation and scale-up of algae biotechnology via (1) design and evaluation of three types of biorefineries aiming to fully exploit algae biomass, (2) definition of an agenda for optimization, and (3) a better understanding of the societal benefits of algae cultivation and use as well as consumers attitude and expectations concerning algae products.

4.2 Dissemination activities

MIRACLES dissemination strategy

The main goal of dissemination in MIRACLES is to promote and raise awareness about the project achievements via effective dissemination and communication within the consortium and with external stakeholders. The main objectives of the dissemination plan in MIRACLES are:

- To enable effective internal communication within the consortium,
- To promote dissemination of the project results at European, international, national, local levels and to reach all target audiences incl. the general public, academia, policy makers, NGO's and industry;
- To facilitate transfer of exploitable results and best practices to end users, mainly in industry.
- To explore potential synergies with related EU projects, incl. SPLASH, D-FACTORY, PUFAchain and BISIGODOS and to perform joint dissemination and training activities with these projects;
- To ensure wide dissemination of project results to reach the highest impact on society;
- To advice policy makers and authorities;
- To inform investors about the commercial opportunities;
- To inform NGO's about the environmental and societal impacts.

Deliverable report D 7.20 (PUBLIC) describes in detail all MIRACLES dissemination and communication activities. In this section, a summary of these activities is presented. ***Table 2*** presents the main target groups and communication channels.

Table 2: *MIRACLES main target groups per communication channel.*

Target Group/Communication Channel	EC	Public Authorities	Industry	Research sector	General public
Website	x	x	x	x	x
Deliverables -restricted	x				
Deliverables -public	x	x	x	x	x
Technical & Scientific Publications	x	x	x	x	
Workshops organized by MIRACLES	x	x	x	x	
Attendance to scientific conferences by partners	x	x	x	x	
Industrial/ Sectorial fairs, workshops, events		x	x		
Stakeholder meetings	x	x	x	x	
Social media			x	x	x
Audiovisual media (videos)	x				x
Posters, roll up, leaflets, etc.		x	x	x	x
Printed & online press					x

Project identity set: logo, brochure and roll-up

A visual identity for the MIRACLES Project was designed. The aim of the logo (specific image with specific colours*;* ***Figure 35***) is to create an easily recognizable "image" of the project, to facilitate its identification in all dissemination activities. In addition a project brochure *(**Figure 36***) and a roll up *(**Figure 37***) were designed and produced by IDConsortium with the collaboration of the consortium. Furthermore templates were produced for posters, presentations and other items.

Figure 35*: MIRACLES Logo*

Figure 36*. MIRACLES brochure*

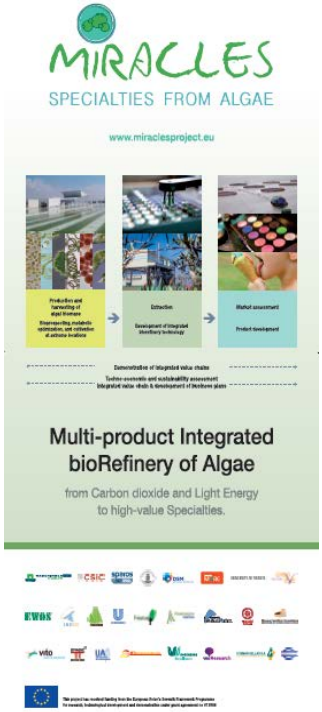

Figure 37: *MIRACLES roll-up*

Project website, internal communication platform and social networks

The project website (http://MIRACLESproject.eu/) was launched in April 2014, and represents the primary source of news dissemination and information about MIRACLES activities (***Figure 38).*** The website includes Web 2.0 features, incl. direct access to the project's Social Networks. To date the website received 36.714 visits and 8.800 new users from all over the world as shown in ***Figure 40.*** The project website will be maintained and updated by IDC until at least 31st December 2019.

Figure 38 *MIRACLES public website home page.*

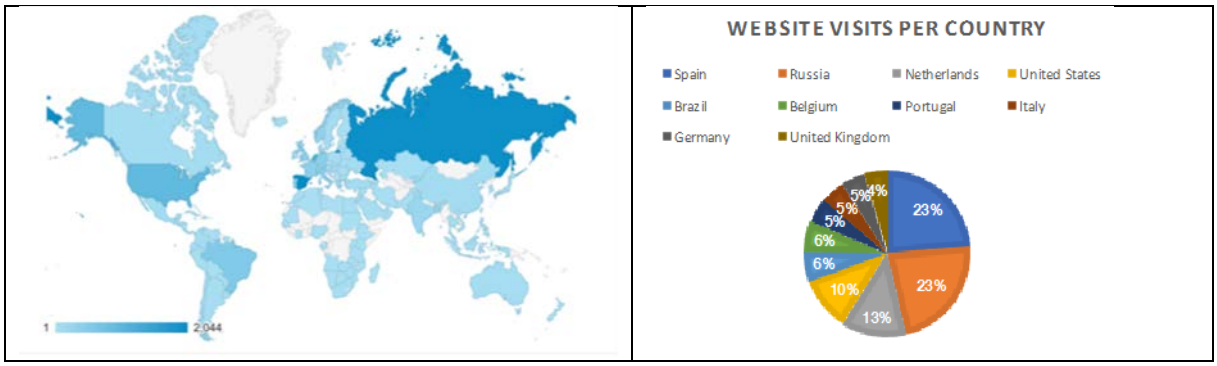

Figure 39. *MIRACLES Website Analysis.*

An Internal communication platform (EMDESK) is accessible (password protected) for project partners and the EC Officer via a link on the web site or directly via https://emdesk.eu. The platform supports document sharing and other facilities.

Social Networks. MIRACLES joined Twitter and Facebook to optimize communication about the project with the partners and with stakeholders. The social media were used to publish short posts on recent MIRACLES activities, conferences where the project was presented, links to related EU projects, videos developed for the project or related to the project and other topics. The overall statistics for MIRACLES social networks since the project start are presented in ***Table 3***.

Table 3. *MIRACLES social media statistics*

		Nov'14	Nov'15	Nov'16	Oct´17
Twitter	Followers	57	107	322	491
	Tweets	5	48	83	147
Facebook	Publications	5	42	90	126
	Likes	32	72	131	205

Videos and TV Shows

In May 2017, a TV documentary "*Green gold in our sea water- Futuris"* was produced about the MIRACLES project by Euronews Television and the European Commission. An estimated 600.000 viewers may have seen this documentary on television and/or on YouTube. In addition partners UiB, UniRes, Sparos and Chimar developed videos about their project activities and results. Partners Fitoplancton and CSIC took part in TV shows promoting the project and algae in general e.g. in food. All MIRACLES videos are compiled in a YouTube Channel specifically created for the project: https://www.youtube.com/playlist?list=PLdtdumBUglfMLJmiCeddm6reLREsXaFS6

Development of Stakeholders list

One of the goals of the project was the identification of relevant contacts and stakeholders in order to empower and improve their overall awareness, and their engagement and participation in the project. For this purpose, a stakeholder list was created for disseminating the project progress, activities and results. The final contacts and stakeholders list contains 280+ contacts, classified per stakeholder group incl. type of industry, academia, public authorities and policy makers, NGOs, press related and general public. A joint stakeholder list was developed with two other FP7 projects in the field of algae: FUEL4ME and SPLASH. In July 2014, the three stakeholder lists were consolidated into a single listing with > 1000 contacts, which was re-defined and its classification made simpler. This new document was shared with the dissemination officers of the two other algae projects.

Newsletters

The 6-monthly newsletter is a tool for engaging stakeholders into the project's results as well as highlighting project meetings, events and other activities. To date 8 newsletters were made available via the project's website. Contacts in the stakeholders list received the newsletters by e-mail.

Scientific publications

To date 18 scientific (peer reviewed) publications by MIRACLES partners highlighting project results have been accepted for publication, 6 of these with open access. The publications are listed in ***Table 5*** "List of Scientific Publications" in section 5.1. Ca. 20 additional peer reviewed publications are in preparation. For update see the project website at : http://miraclesproject.eu/articles.php

Conferences and events

MIRACLES partners have participated in a range of events to disseminate project results via lectures and poster presentations (listed in table 7 "Dissemination Activities" in section 5.1). In addition, several events were held where the MIRACLES consortium partners participated actively in the organization:

- Bio Economy Investment Summit: 9th-10th of November, 2015, Brussels, BE
  MIRACLES was represented in the conference and had a stand presenting project information and algae products. Several contacts were established with interested parties in various areas.
- Conference: European Roadmap for an algae based Industry 6-8 April 2016, Olhao, PT.
  This conference (http://eualgaeroadmapconference.eu/42/) was co-organized by several European projects in the algae field (incl. MIRACLES Fuel4Me, Splash, AlgaeCluster,D-Factory,...) (***Figure 40).*** Partners presented project results via lectures and posters.

Figure 40 *Participants conf. European Roadmap for an algae based Industry 6-8 April 2016, Olhao, PT.*

- Conference Algae Biorefineries for Europe: 17-18 October, 2017, Brussels, BE.

A major highlight was the conference *"Algae Biorefineries for Europe: towards a sustainable economy"* held in Brussels, BE, on 17th and 18th October 2017 (***Figure 41***). This conference was co-organized by the consortia of four major European collaborative R&D projects on microalgae biorefinery: Bisigodos, D-Factory, MIRACLES and PUFAChain to showcase their progress on the sustainable use of microalgae. More than 100 people attended the conference with representatives from industry, policy makers and researchers across Europe and beyond. Apart from the lectures given by partners of the consortium, MIRACLES products and posters were exhibited during the networking sessions. Conference documents (lectures, posters) are available at www.algaebiorefineryconference.eu.

Training and Exchange of researchers

During the project many RTD and industrial partners worked with students in MSc and BSc internships and PhD projects. Multiple partners participated in the conference EU Algae Industry Roadmap conference (April 2016) and Algae Biorefineries for Europe Conference (Oct. 2017). Many partners contributed to training and exchange of students and researchers and interacted with students via school visits, science festivals and other events.

"Biorefinery summer school" on 28-31 August 2017, Wageningen, NL.

The Biorefinery summer school was held August 28 – 31st, 2017 in Wageningen, NL. This *2nd International Course on Microalgae Biorefinery* was organized by the WUR VLAG Graduate school in collaboration with MIRACLES. The course had 20 participants from 12 nationalities. The course was aimed at academics as well as professionals from industry, and intended to provide the essential skills for designing optimal microalgae-based biorefineries, from unit operations to the entire process chain, and to be able to address the present bottlenecks. Speakers from industry highlighted the industrial framework of algae biorefinery including MIRACLES partners Carlos Unamunzaga, CEO Fitoplancton and Philippe Willems, Value For Technology (VFT); and in addition Reza Ranjbar, CTO of AlgaeBiotech.

Figure 41. *Impressions from the conference "Algae Biorefineries for Europe" in Brussels, BE, on 17th and 18th October 2017.*

Conclusions dissemination and communication

The dissemination efforts in the framework of MIRACLES can be considered as successful. Dissemination will be ongoing in the coming period. The following conclusions and lessons have been compiled from the MIRACLES communication and dissemination activities:

1. Communication and dissemination is a very important activity in innovation projects to implement the advances in science and innovation. All partners should be aware of the importance of this and actively cooperate in these activities. The dissemination officer of every project should know all partners and how to motivate them to join this activity.
2. It is recommended to have a team of 3-4 people from different partners and with different skills (scientific+ industrial/ technological+ a partner with knowledge in consumer behaviour) responsible for the communication and dissemination.
3. Scientific and technical communication is important, but the communication to the general public (undergraduate, households, university students and population in general) is also essential in order to educate and raise awareness about how science and innovation can improve their life. Nowadays, social networks and audio-visual media are a powerful tool for this.
4. The dissemination and communication team of every project should be aware of the new and innovative communication channels because they are continuously changing.
5. It is very important to measure the impact of the communication and dissemination and to react quickly with any deviation of the initial plan.

4.3 Marketing and business plan

Based on the project results, the consumer acceptance screening, and an analysis of main consumer trends, partner VFT developed a positioning and marketing strategy for MIRACLES technologies and products and edited a marketing and business plan.

The financial analysis in the Business plan clearly indicates that multiproduct strategies are close to economic feasibility at a 10,000 ton scale. (See paragraph 3.6 for details). Conclusions:

- The results indicate a profitable business potential for three developed multiproduct scenarios. A 10.000 T multiproduct microalgae biorefinery is potentially profitable
- Single product biorefineries of similar sizes however are far from profitable
- The profitability of multiproduct scenarios is caused by new cost reduction technologies developed within MIRACLES, combined with value creating new application developments and an innovative positioning strategy.

The main stakeholder, when considering marketing microalgae products, is the final consumer, pulling the whole value chain. In scope of an emerging microalgae business, a focus group of consumers was identified, being the LOHAS (Lifestyles Of Health And Sustainability) community, representing 25% of the European households. These 'Lohasians' particularly prefer products with a positive impact on their health and wellbeing, products that are sustainable and aesthetic, products giving them a 'good

conscience'. They are typically mid-thirties to late-fifties, well educated and with above average income.

How do these LOHAS consumers look at microalgae? The MIRACLES consortium performed a preliminary consumer acceptance study indicating a positive attitude towards microalgae as a natural and nutritional element in the food chain, environmental aspects such as wildlife/nature preservation (no fish meal/oil needed), local production, no need for arable land. Concerns are related to taste and smell issues, the risk of toxins produced by microalgae, the possible accumulation of contaminants and possible adverse reactions on human skin. Knowing these element allows for a focused quality control and communication towards the consumers.

The new positioning strategy first focuses on the consumer, identified the LOHAS (=Lifestyles Of Health And Sustainability) consumer as most promising focus group and proposes a balanced message based on rational (the functionality), emotional (the aesthetics and organoleptics) and intuitive (the personal values) properties of microalgae ingredients and the end products in which these are processed. This needs to be translated into an honest and well documented message as a basis for communication with the consumers. The final result of this is an increased consumer value for microalgae products. In combination with cost reduction technologies developed within the MIRACLES project, this may lead to profitable business cases.

4.4 Exploitation of results

Analysis of foreground results

MIRACLES generated 85 distinctive results on average at TRL 4-5, almost evenly split between RTD and industrial partners, almost evenly split between strain prospection, production processes and application development. All exploitable results are listed in Section 5.2 Exploitable foreground.

In total 85 exploitable results were reported by the MIRACLES consortium, covering 6 Work Packages.

- 44 results are attributed to RTD partners, 41 to industrial partners, almost a 50:50 ratio.
- 1/3 on strain prospection, 1/3 on production processes and 1/3 on applications (rounded).
- 46 results will be further researched by the respective owners, both industrial and RTD.
- 38 results were reported in 15 peer reviewed publications and 25 forthcoming scientific publications (published and in preparation) by the RTD partners of the project.
- 24 results are leading to potential commercial activities for industrial partners.
- 18 results are open for technology transfer to third parties (within our outside the consortium). Mainly industrial partners will be involved in this exploitation route.
- 1 patent has been filed by CSIC, who now formally proposes their extraction technology for licensing. Partners within the consortium already indicated their interest.
- 5 more results covering 2 applications are being investigated regarding their patentability, both by industrial partners in the end-user field.
- 1 technology (CO2 capture equipment by UT) will possible be embedded in a spin-off company. Partner VFT will assist UT in this post-MIRACLES exercise.

- 2 possible spin-offs may be created based on the results obtained in MIRACLES, one based on a process, the other on an application.

A summary of the acquired foreground results is presented in ***Figure 42*** below.

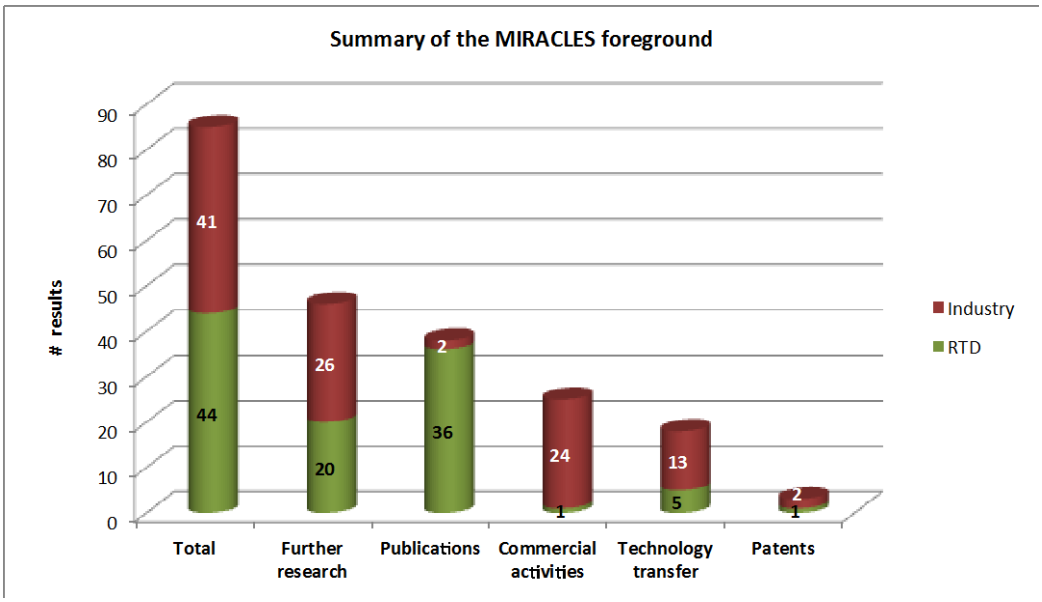

Figure 42. *Summary of the MIRACLES foreground results*

Maturity of the results: TRL analysis

Assigning a Technology Readiness Level or TRL to each result gives an image of the maturity of those results and by extension of the MIRACLES Project. For a good understanding, the TRL definitions as used by the European Commission as presented in ***Table 4*** below.

Table 4. *Technology Readiness Levels*

Technology Readiness Levels

TRL 0: Idea. Unproven concept, no testing has been performed.
TRL 1: Basic research. Principles postulated and observed but no experimental proof available.
TRL 2: Technology formulation. Concept and application have been formulated.
TRL 3: Applied research. First laboratory tests completed; proof of concept.
TRL 4: Small scale prototype built in a laboratory environment ("ugly" prototype).
TRL 5: Large scale prototype tested in intended environment.
TRL 6: Prototype system tested in intended environment close to expected performance.
TRL 7: Demonstration system operating in operational environment at pre-commercial scale.
TRL 8: First of a kind commercial system. Manufacturing issues solved.
TRL 9: Full commercial application, technology available for consumers.

It is generally accepted that at the beginning of the project, the TRL was around 2/3. Some concepts were formulated (TRL 2) such as the foam bed reactor, the use of algae in plant growth promoters, the possibility to have antimicrobial peptides in microalgae protein hydrolysates, etc. Some technologies had a proof-of-concept (TRL 3) such as the immersed membranes, the sequential extraction process, the presence of anti-oxidant compounds. Finally also some initial TRL 4 technologies such as the specific microalgae compounds for aquafeed studied in MIRACLES, the growing processes, compounding microalgae in thermoplastics. Reviewing the results obtained in MIRACLES by their present TRL leads to the distribution presented in ***Figure 43*** below.

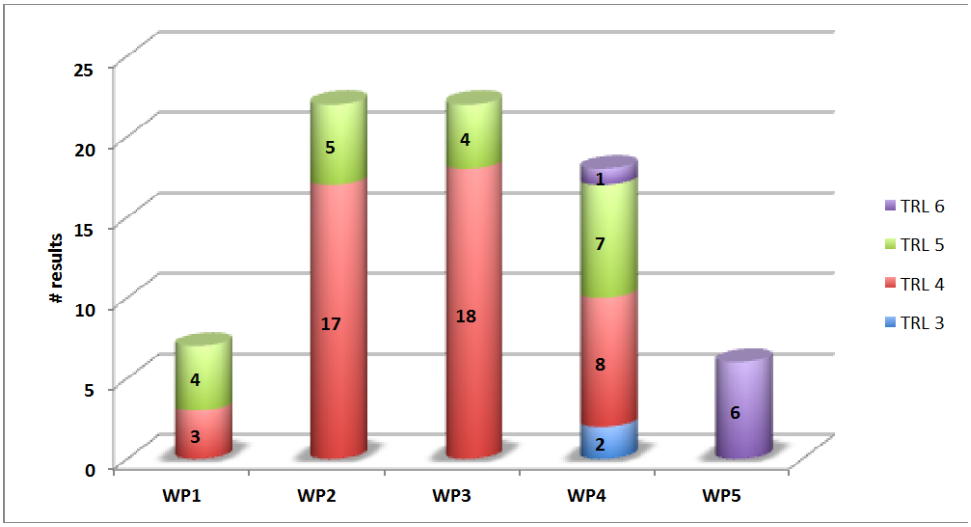

Figure 43. *TRL level of forgeround developed in the MIRACLES project*

MIRACLES technologies are now at a TRL level of 4 and 5, with some exceptions in both directions. This is an increase of at least 2 TRL points compared to the starting position. The overall results are very promising, but only validated at a TRL 4-5 level and need further research and demonstration to be boosted to TRL 7-9. Also the ideal plant size according to the selected product mix and a detailed business modelling needs to be defined/refined.

More interesting then this quantitative analysis is the qualitative one. New technologies have been explored and validated with a potential for significant cost reduction; new applications have been validated with a potential of premium value creation. This widens the gap between cost and revenue, creating opportunities for economically viable microalgae biorefineries.

Regarding IP, 1 patent has been filed, 2 other are in the process of being filed. 25% of the results will be considered for further commercial valorisation by the owners, often protected by some trade secret IP. This may lead towards the creation of new companies, exploiting this results commercially.
Most important regarding the exploitation of the results, is the broad support to one or more follow-up project(s). Such projects will be designed by a 'core team' of MIRACLES partners.

Impact of exploitable foreground

The finality of MIRACLES is to develop technologies to substantially reduce the cost of microalgae (products) production together with application research to increase the value of these algae products.

In this section, those results with potential high impact on cost reduction and value creation are summarized.

Cost reduction foreground

Next to a set of useful tools (such as the molecular tools for real-time monitoring of valuable bioproducts during microalgae production; the study on cell disruption etc.) 4 technologies have the potential to create a step change in the algae production cost:

1. Technology for CO_2 capture from ambient air allowing to install a microalgae production plant independently from a CO_2 source and at competitive CO_2 cost;
2. A foam-bed photobioreactor, increasing the algae concentration from 2 - 3 g/l in a conventional flat panel reactor to 30g/l, with significantly improved gas transfer, CO_2 utilisation and >10 fold reduced energy requirement for reactor operation and harvesting.
3. A submerged membrane system allowing for effective pre-concentration of the algae biomass and almost quantitative recycling of the growth medium, reducing energy use and costs.
4. A combined set of high pressure extraction techniques (pressurised liquid extraction, supercritical fluid extraction and gas-expanded liquid extraction) allowing for an effective extraction of pigments, lipids, leaving a protein-rich residue. This platform is patented.

Value creation foreground

Novel applications for microalgae in the 'high value' segment have been successfully validated:

1. A positive effect of microalgae in fish diets on skin pigmentation and quality of the meat, making the fish more desirable for consumers.
2. Anti-microbial properties of algae peptides with anti-spoilage effect in cosmetics and food
3. Plant-growth promoting effects of microalgae extracts, leading to better looking plants, longer pot-life for fresh spices, higher growth rate for cereals, etc. Patent pending.
4. Biodegradable thermoplastic compounds containing microalgae extracts as slow release properties promoting plant growth and biodiversity. Patent pending.
5. Incorporation of algae whole cells or residues as aesthetic fillers in thermosetting systems. High value applications in decorative elements such as table-tops and lamp covers.
6. New business model to valorise microalgae with maximal consumer value.

Valorisation of foreground by industrial partners

Industrial partners in MIRACLES have achieved positive results with exploitation potential. But also RTD partners generated results that are possibly exploitable by the industrial partners**.**
Active exploitation of the foreground results is undertaken by the involved industrial partners in collaboration with the projects IPR and Exploitation Officer VFT. 13 industrial partners have defined exploitation/ valorisation intentions for one or more foreground results resulting from MIRACLES.

Regarding IP strategy related to the valorisation of the results, CSIC **patented** their sequential high pressure extraction process and offered it for licensing. Possible interest from ET. Five other results (on the preservative properties of microalgae peptides and the plant growth promoting effects) are currently being investigated for **2 more possible patents**.

The positioning strategy proposed by VFT will be available for bio-economy actors and valorised on a consulting base by VFT. All other results will be kept a **trade secret** and valorisation schemes, including partnership with relevant third parties will be evaluated by the respective result owners.

Finally, the **creation of new commercial ventures** to exploit results is under consideration.

4.5 Contacts

Project website: *http://MIRACLESproject.eu/*

Project Coordinator: Hans Reith, Wageningen University, Agrotechnology & Food Sciences.
E-mail: hans.reith@wur.nl
Tel. +31 (0) 317 485228

IPR and Exploitation Officer: Philippe Willems, Value for Technology (VFT)
E-mail: phw@value-for-technology.be
Telephone: +32 2 759 33 10 / Mobile: +32 475 801 602

Dissemination Officer: Macarena Sanz, IDConsortium
E-mail: msanz@idconsortium.es
Mobile: +34 619856468

EC Project Officer: Andrea GRISORIO, European Commission, DG Research & Innovation. F2. Bio-based products and processing.
e-mail: Andrea.GRISORIO@ec.europa.eu ; Tel.: +32 229-86533

4.6 CONSORTIUM partners

NB: Clicking on the partner name while holding the CTRL button will take you to the partners website.

P1a – Wageningen University - BioProcess Engineering (WU-BPE, NL)

P1b – Wageningen University-Biobased Chemistry and Technology (WU-BCT, NL)

P2 – University of Las Palmas de Gran Canaria (FCPCT), Spanish Bank of Algae (BEA, ES)

P3 – University of Twente (UT, NL)

P4 – University of Bergen, Department of Biology (UiB, NO)

P5 – Universidad de Huelva (UHU, ES)

P6 – Universidad de Antofagasta (UA, CL)

P7 – Wageningen Food & Biobased Research/ Stichting Dienst Landbouwkundig Onderzoek (WFBR/DLO NL)

P8 – CSIC, Agencia Estatal Consejo Superior De Investigaciones Científicas (CIAL), Foodomics laboratory (CSIC, ES)

P9 – Vlaams Instituut voor Technologisch Onderzoek - (VITO, BE)

P10 – Cargill / EWOS Innovation AS (EWOS, NO)

P12 – Fitoplancton Marino S.L. (FITO, ES)

P13 – SPAROS LDA (SPAROS, PT)

P14 – Rodenburg Biopolymers (BIOPOL, NL)

P15 – IMEnz Bioengineering (IMENZ, NL)

P16 – Chimar Hellas (CHIMAR, GR)

P17 – Value for Technology - (VFT, BE)

P18 – Natac Biotech (NATAC, ES)

P19 – Nova-Institut – (NOVA, DE)

P20 – ID Consortium (IDC, ES)

P21 – Eco Treasures (ET, BE)

P22 – CropEye (CE, NL)

P23 – Unilever Research & Development Vlaardingen – (URDV, NL)

P24 – DSM Food Specialties BV (DSM, NL)

P25 – Thomas More Kempen – (TMUC, BE)

P26 – UniResearch AS Bergen – (UniRes, NO)

5 Use and dissemination of foreground

The projects dissemination activities are presented in section 4.2.

5.1 Dissemination measures relating to foreground

This section describes the dissemination measures, including scientific publications relating to foreground.

Table 5 (follows Template A1) and presents a list of all scientific (peer reviewed) publications relating to the foreground of the project.

The table is cumulative, and show all publications from the beginning until after the end of the project. Updates will be provided on the project website http://miraclesproject.eu/

5.1.1 Scientific publications

Table 5 presents a list of peer reviewed publications by MIRACLES partners relating to the foreground of the project. Per July 2018 18 scientific (peer reviewed) publications by MIRACLES partners highlighting project results have been accepted for publication, 6 of these with open access. Up to 20 additional peer reviewed publications are in preparation.

The publications will be updated on the project website: http://miraclesproject.eu/articles.php

According to clause nº 39 in the Grant Agreement, the involved partners have made and are making efforts to provide open access. This is indicated in the last column of Table 5.

Table 5: *MIRACLES peer reviewed publications to date. Status per July 2018.* ***An updated list of publications is available on the project website: http://miraclesproject.eu/articles.php***

eA1-LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series// Name of the conference**	**Number, date or frecuency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers(if available)**	**Is/Will open access provided to this publication?**
1	***Green downstream processing of Isochrysis galbana: A step towards microalgal biorefinery.***	Bienvenida Gilbert-López, José A. Mendiola, Javier Fontecha, Lambertus A. M. van den Broek, Lolke Sijtsma, Alejandro Cifuentes, Miguel Herrero, Elena Ibáñez	Green Chemistry	No. 17/2015	Royal Society of Chemistry	UK	2015	pp. 4599-4609	DOI:10.1039/C5GC01256B	Yes
2	***Green foodomics. Towards a cleaner scientific discipline***	Gilbert-López, B., Mendiola, J.A., Ibáñez, E.	TrAC-Trends in Analytical Chemistry	June 2017	Elsevier	UK	2017	NA	DOI: 10.1016/j.trac.2017.06.013	No
3	***The potential of optimized process design to advance LCA performance of algae production systems.***	A.J.B. van Boxtel, P. Perez-Lopez, E. Breitmayer, P.M. Slegers	Applied Energy	no. 2/2015	Elsevier	NA	2015	NA	DOI: 10.1016/j.apenergy.2015.01.036	No
4	***A liquid foam-bed photobioreactor for microalgae production***	Agnes Janoska, Packo P. Lamers, Alex Hamhuis, Yorick van Eimeren, Rene H. Wijffels, Marcel Janssen	Chemical Engineering Journal	No. 313/2017	Elsevier	NA	2017	pp. 1206-1214	DOI: 10.1016/j.cej.2016.11.022	No

eA1-LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series// Name of the conference**	**Number, date or frecuency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers(if available)**	**Is/Will open access provided to this publication?**
5	***Biorefinery of microalgal soluble proteins by sequential processing and membrane filtration.***	Safi C, Olivieri G, Campos RP, Engelen-Smit N, Mulder WJ, Van den Broek LAM, Sijtsma L	Bioresource Technology	No. 225/2017	Elsevier	NA	2017	pp. 151-158	DOI: 10.1016/j.biortech.2016.11.068.	Yes
6	***Energy consumption and water-soluble protein release by cell wall disruption of Nannochloropsisgaditana.***	Safi C, CabasRodriguez L, Mulder WJ, Engelen-Smit N, Spekking W, Van den Broek LAM, Olivieri G, Sijtsma L	Bioresource Technology	No. 239/2017	Elsevier	NA	2017	pp. 204-210	DOI: 10.1016/j.biortech.2017.05.012	No
7	***Green compressed fluid technologies for downstream processing of Scenedesmus obliquus in a biorefinery approach***	B. Gilbert-López, J. A. Mendiola, L., van den Broek, B.Houweling-Tan, L.Sijtsma, A. Cifuentes, M. Herrero, E. Ibañez	Algal Research	No. 24/2016	Elsevier	NETHER-LANDS	2016	pp. 111-121	DOI: 10.1016/j.algal.2017.03.011	No
8	***Development of new green processes for the recovery of bioactives from Phaeodactylumtricornutum***	B. Gilbert-López, A. Barranco, M. Herrero, A. Cifuentes, E. Ibáñez	Food Research International	NA	Elsevier	NETHER-LANDS	2016	pp. 1056-1065	DOI: 10.1016/j.foodres.2016.04.022	No
9	***New green approaches for the selective extraction of bioactive compounds from natural sources: Nannochloropsisgaditana as a case study***	A. P. Sánchez-Camargo, N. Pleite, M. Herrero, E. Ibáñez*, B. Gilbert-López	Journal of Supercritical Fluids	No. 128 /2016	Elsevier	NETHER-LANDS	2016	pp.112-120	DOI: 10.1016/j.supflu.2017.05.016	No

eA1-LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series// Name of the conference**	**Number, date or frecuency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers(if available)**	**Is/Will open access provided to this publication?**
10	***Adsorbent-assisted supercritical CO2 extraction of carotenoids from Neochlorisoleoabundans paste***	Reyes, F.A., Mendiola, J.A., Suárez-Alvarez, S., Ibañez, E., Del Valle, J.M.	Journal of Supercritical Fluids	No. 112/2016	Elsevier	NETHER-LANDS	2016	pp. 7-13	DOI: 10.1016/j.supflu.2016.02.00	No
11	***Gas expanded liquids and switchable solvents***	Herrero, M., Mendiola, J.A., Ibáñez, E.	Current Opinion in Green and SustainableChemistry	No. 5/ March2017	Elsevier	NETHER-LANDS	2017	pp. 24-30	DOI: 10.1016/j.cogsc.2017.03.008	No
12	***Bioprospecting North Atlantic microalgae with fast growth and highpolyunsaturated fatty acid (PUFA) content for microalgae-based technologies***	Pia Steinrücken, Svein Rune Erga, Svein Are Mjøs, Hans Kleivdal, Siv Kristin Prestegard	Algal Research	No. 26/2017	Elsevier	NA	2017	pp. 392-401	DOI: 10.1016/j.algal.2017.07.030	Yes
13	***Stability of a Benzyl Amine Based CO2 Capture Adsorbent in View of Regeneration Strategies***	Q.Yu, J.de la P. Delgado, R.Veneman, D.W.F. Brilman	Industrial & Engineering Chemistry Research	No. 56/2017	American Chemical Society	Washington D.C., US	2017	pp. 3259-3269	DOI: 10.1021/acs.iecr.6b04645	Yes
14	***Design Strategy for CO2 Adsorption from Ambient Air Using a Supported Amine Based Sorbent in a Fixed Bed Reactor***	Q.Yu, D.W.F. Brilman	Energy Procedia	No. 114/2017	Elsevier	Amsterdam NL	2017	pp. 6102-6114	DOI: 10.1016/j.egypro.2017.03.1747	Yes
15	***Comparing EPA production and fatty acid profiles of three Phaeodactylum tricornutum strains under western Norwegian climate conditions***	Pia Steinrücken, Siv Kristin Prestegard, Jeroen De Vree, Julia E. Storesund, Bernadette Pree, Svein Are Mjøs, Svein Rune Erga,	Algal Research	No. 30/2018	Elsevier	NA	2018	pp. 11-22	DOI: 10.1016/j.algal.2017.12.001	Yes

eA1-LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
No.	**Title**	**Main author**	**Title of the periodical or the series// Name of the conference**	**Number, date or frecuency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifiers(if available)**	**Is/Will open access provided to this publication?**
16	***Effect of nitrogen addition during the night on lipid productivity of nitrogen starved Nannochloropsis gaditana***	Jorijn H. Janssen*, Jens Kastenhofer, Jacob A. de Hoop, Packo P. Lamers, René H. Wijffels and Maria J. Barbosa	Algal Research	Volume 33, July 2018,	Elsevier		2018	Pages 125-132	https://doi.org/10.1016/j.algal.2018.05.009	
17	***Surfactant selection for a liquid foam-bed photobioreactor***	Agnes Janoska and María Vázquez, Marcel Janssen, Rene H. Wijffels, María Cuaresma, Carlos Vílchez	Biotechnology Progress Journal (17/01/18).	2017	Wiley On Line	-	2018	-	|https://doi.org/10.1002/btpr.2614	
18	***Improved liquid foam-bed photobioreactor design for microalgae cultivation***	Agnes Janoska, Robin Barten, Sam de Nooy, Piotr van Rijssel, René H. Wijffels, Marcel Janssen	Algal Research	Volume 33, July 2018,	Elsevier		2018	Pages 55-70	https://doi.org/10.1016/j.algal.2018.04.025	

5.2 Exploitable foreground

5.2.1 Patent applications

Template B1: List of applications for patents, trademarks, registered designs, etc.					
Type of IP Rights	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	No		ES201730896	Procedimiento para la obtención secuencial de compuestos de alto valorañadido a partir de biomasa húmeda	CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS (CSIC); https://flucomp.es/imagenes/media/13.pdf

NB: Several other foreground results are currently being investigated for 2 more possible patents.

5.2.2 Exploitable foreground

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation	Cultivation strategy to induce accumulation of oils/TAG	YES		Production process	A1.2.9 A1.6.1 M74.9.0			FITO (owner) WU
Commercial exploitation	Development of tools to monitor and optimize the microalgae production process	YES		Control system	A1.2.9 A1.6.1 M74.9.0		Licensing	FITO (owner)
Commercial exploitation	Equipment to concentrate CO2 from ambient air	NO		CO_2 capture equipment	A1.2.9 A1.6.1 C28.9.9		Licensing / Spin-off	UT (owner)
General advancement of knowledge	Functional design of a liquid foam-bed reactor photobioreactor	NO		Photobioreactor	A1.2.9 A1.6.1 C28.9.9			WU (owner)
General advancement of knowledge	Polymer-based component that facilitates the foam-breaking in a foam-bed cultivation system.	NO		Processing aid	A1.2.9 A1.6.1 M74.9.0			UHU (owner)
Commercial exploitation	Submerged membranes technology (MAF) for integrated algae pre-harvesting and water recirculation	NO		Harvesting equipment	A1.2.9 A1.6.1 C28.9.9		Licensing	VITO (owner)
General advancement of knowledge	Medium recycling process	NO		Production process	A1.2.9 A1.6.1 M74.9.0			TMUC (owner) VITO
Commercial exploitation	Bioprospecting of microalgae: input for screening criteria	NO		List of criteria	A1.2.9 A1.6.1 M74.9.0			All WP4
General advancement of knowledge	11 strains with proteins, carbohydrates and lipids	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			UA (owner)
General advancement of knowledge	Halothece sp. with phycocyanin for solar cells	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			UA (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	3 strains with pigments phycocyanin, phycoerythrin and fucoxanthin	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			UA (owner)
General advancement of knowledge	154 clonal strains of 9 different class	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			FCPCT-BEA (owner)
General advancement of knowledge	24 tested strains indoor & outdoor	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			FCPCT-BEA (owner)
General advancement of knowledge	Screening pipeline for bioprospecting omega-3 rich algae	NO		Methodology	A1.2.9 A1.6.1 M74.9.0			UiB (owner) UniRes
General advancement of knowledge	Interesting strains in Biobank	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			UniRes (owner) UiB
General advancement of knowledge	Biomass (*P.tricornutum* B58) produced for IMenz, CE, CHIMAR	NO		Algae biomass	A1.2.9 A1.6.1 M74.9.0			UiB (owner) UniRes
General advancement of knowledge	5 tested strains in small outdoor PBR (GWP)	NO		Algae strains collection	A1.2.9 A1.6.1 M74.9.0			FCPCT-BEA (owner)
Commercial exploitation	Metabolic modeling and studies to maximize product yields for target products	YES		Production know-how	A1.2.9 A1.6.1 M74.9.0		Licensing	FITO (owner)
General advancement of knowledge	Effect of nightly addition of nitrogen to a nitrogen starved culture.	NO		Production know-how	A1.2.9 A1.6.1 M74.9.0			WU (owner)
General advancement of knowledge	Optimal biomass concentration at the start of nitrogen starvation for maximal TAG yield.	NO		Production know-how	A1.2.9 A1.6.1 M74.9.0			WU (owner)
General advancement of knowledge	Fatty acid pathway analysis using carbon-13	NO		Production know-how	A1.2.9 A1.6.1 M74.9.0			WU (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Transcriptome data analysis during nitrogen starvation focused on the fatty acid accumulation	NO		Production know-how	A1.2.9 A1.6.1 M74.9.0			WU (owner)
General advancement of knowledge	Biochemical analysis of strains from bioprospection	NO		Analytical data	A1.2.9 A1.6.1 M74.9.0			UA (owner) CSIC BEA
General advancement of knowledge	Integration and comparison of acquired data	NO		Analytical data	A1.2.9 A1.6.1 M74.9.0			FCPCT-BEA (owner)
General advancement of knowledge	Benchmark of biochemical characterisation data of microalgal species	NO		Analytical data	A1.2.9 A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Standardised methods to provide a detailed biochemical characterisation for microalgae.	NO		Analytical method	A1.2.9 A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Optimisation of cell disruption methods	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Investigating the efficiency of Pulsed Electric Field (PEF) on breaking the resistant cell wall	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Enzymes for cell disruption	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner) DNL DSM
Commercial exploitation	Several commercial DSM enzymes successfully tested for mild disruption of algae biomass	YES		Biorefining method	A1.6.3 M74.9.0			DSM (owner)
General advancement of knowledge	Application of microorganisms for the disruption of algae	NO		Biorefining method	A1.6.3 M74.9.0			IMENZ (owner)
General advancement of knowledge	Explosive high pressure decompression in CO2 medium	YES		Biorefining method	A1.6.3 M74.9.0			ET (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	A filtration process established for Chlorophyll-free protein solution	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	A filtration process established for Chlorophyll-free peptide solution	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Repeated washing after centrifugation of disrupted cells increased the protein yield.	NO		Biorefining method	A1.6.3 M74.9.0			WFBR (owner)
General advancement of knowledge	Sequential extraction process for dry and wet algae biomass	NO		Biorefining method	A1.6.3 C10.4.1 M74.9.0			CSIC (owner) WU
General advancement of knowledge	Sequential extraction process for dry algae biomass	NO		Biorefining method	A1.6.3 C10.4.1 M74.9.0			CSIC (owner)
General advancement of knowledge	Selective extraction of fucoxanthin from microalgal biomass	NO		Biorefining method	A1.6.3 C10.4.1 M74.9.0			CSIC (owner)
General advancement of knowledge	Extraction of antioxidants from microalgal biomass	NO		Biorefining method	A1.6.3 C10.4.1 M74.9.0			CSIC (owner)
General advancement of knowledge	Extraction of carotenoids and polar lipids from microalgal biomass	NO		Biorefining method	A1.6.3 C10.4.1 M74.9.0			CSIC (owner)
Commercial exploitation	Oil extraction process upscaling and extractor design	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0			ET (owner)
Commercial exploitation	Refining of the algae oil	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0		Licensing	ET (owner)
Commercial exploitation	Recuperation of carotenoids out of bleaching earth to use in end applications	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0		Licensing	ET (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation	Sequential extraction process for algae wet biomass optimised	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0		Patent application & licensing	CSIC (owner)
Commercial exploitation	Sequential process for lipids + carotenoids from alga biomass optimised	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0		Licensing	CSIC (owner) ET
Commercial exploitation	Sequential process for lipids + carotenoids from algae biomas optimised	YES		Biorefining method	A1.6.3 C10.4.1 M74.9.0		Licensing	CSIC (owner)
General advancement of knowledge	Emulsification results and Mayo product application using protein fractions from microalgae	YES		Processed food product	C10.8.9			URDV (owner)
General advancement of knowledge	Total fatty acid, TAG and Polar lipid analysis of crude oil fractions	YES		Analysis	C10.4.1 M74.9.0			URDV (owner)
Commercial exploitation	Nutritional value of phototrophic EPA algae in small fish growth and digestibility screening trials for salmon.	YES		Aquafeed recipe	A3.2.1 A3.2.2 C10.9.1			EWOS (owner) SPAROS FITO ET
Commercial exploitation	Process for direct incorporation of wet microalgae biomasses in extruded aquafeeds	YES		Aquafeed recipe	A3.2.1 A3.2.2 C10.9.1 C10.9.2			SPAROS (owner)
Commercial exploitation	Bio-functional role of microalgae biomasses on the immune response and skin pigmentation in farmed fish	YES		Aquafeed recipe	A3.2.1 A3.2.2 C10.9.1			SPAROS (owner) FITO
General advancement of knowledge	Bio-preservatives from microalgae for control of (food/cosmetics) spoilage related microorganisms	YES		Bio-preservative	C10.8.9 C20.4.2 C20.5.9		Evaluation of patentability	IMENZ (owner) URDV
Commercial exploitation	Antioxidant peptides from different algae	YES		Antioxidant	C10.8.9 C20.4.2 C20.5.9		Licensing	IMENZ (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Commercial exploitation	Two cosmetic prototypes (facial serum and facial cream) including fucoxanthin-rich extract from microalgae with proven activity against ROS (antioxidant, antiaging, sun protection)	YES		Cosmetic facial cream	C20.4.2			NATAC (owner) CSIC FITO
Commercial exploitation	Production of PF resins using algae-derived materials and for the application of such resins as binders in the manufacture of composite wood panels.	YES		Resin and wood panels	C20.5.2 C16.2.1		Licensing	CHIMAR (owner)
Commercial exploitation	Production of non-formaldehyde coatings using algae-derived materials and for the application of such coatings as finishing materials in the manufacture of composite wood panels.	YES		Coating	C20.3.0		Licensing	CHIMAR (owner)
Commercial exploitation	Incorporation of microalgae as aesthetic filler in thermosetting resins	YES		Coating	C20.3.0			VFT (owner)
Commercial exploitation	Solanyl/algae compounds, products, growing pots and plant protection crates	YES		Plastic compound	C22.2.9			BIOPOL (owner)
General advancement of knowledge	Knowledge regarding the interaction of algae and Bioplastic	YES		Know-how	C22.2.9 M74.9.0			BIOPOL (owner)
General advancement of knowledge	Knowledge regarding behavior of algae in Bioplastic	YES		Know-how	C22.2.9 M74.9.0			BIOPOL (owner)
General advancement of knowledge	Knowledge regarding physical property changes in Solanyl regarding the addition of algae.	YES		Know-how	C22.2.9 M74.9.0			BIOPOL (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Knowledge regarding the influence of algae-solanyl compounds on plants	YES		Know-how	A1.6.1 C20.1.5 C22.2.9 M74.9.0		Evaluation of patentability	BIOPOL (owner) CE
Commercial exploitation	Anti stress agent for growing plants	YES		Plant growth promoter	A1.6.1 C20.1.5 M74.9.0		Evaluation of patentability / Licensing	CE (owner)
Commercial exploitation	Anti ageing agent for growing plants	YES		Plant growth promoter	A1.6.1 C20.1.5 M74.9.0		Evaluation of patentability / Licensing	CE (owner)
Commercial exploitation	Nitrogen uptake improver for growing plants	YES		Plant growth promoter	A1.6.1 C20.1.5 M74.9.0		Evaluation of patentability / Licensing	CE (owner)
General advancement of knowledge	Market database	YES		Market know-how	M73.2.0			VFT (owner)
Commercial exploitation	Market positioning strategy	NO		Market know-how	M73.2.0			VFT (owner)
Commercial exploitation	Production of Nannochloropsis enriched high EPA in TAG form	YES		Microalgae	A1.2.9 A1.6.1			FITO (owner)
Commercial exploitation	Aquafeed applications validated on pilot scale	YES		Aquafeed recipe	A3.2.1 A3.2.2 C10.9.1 C10.9.2			EWOS (owner) SPAROS (owner)
Commercial exploitation	Pilot scale validation of facial cream	YES		Cosmetic facial cream	C20.4.2			NATAC (owner) CSIC FITO
Commercial exploitation	Solanyl/Algae compounds and products validated on pilot scale	YES		Plastic compound	C22.2.9			BIOPOL (owner)
Commercial exploitation	Incorporation of microalgae as aesthetic filler in thermosetting resins validated on pilot scale	YES		Coating	C20.3.0			VFT (owner)

Type of Exploitable Foreground	Description of exploitable foreground	Confi-dential	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Demonstration of selected integrated value chains	YES		Know-how	A1.2.9 A1.6.1 A1.6.3 M74.9.0			FITO (owner)
General advancement of knowledge	Conceptual process design models of integrated multi-product biorefinery plant	NO		Know-how	A1.2.9 A1.6.1 A1.6.3 M74.9.0			WU (owner) NOVA
General advancement of knowledge	Evaluation of integral value chain	NO		Know-how	A1.2.9 A1.6.1 A1.6.3 M74.9.0			WU (owner) NOVA
Commercial exploitation	LCA of different biorefinery types	NO		Know-how	A1.2.9 A1.6.1 A1.6.3 M74.9.0			NOVA (owner)
Commercial exploitation	Explorative study on consumer acceptance algae-based products in food, aquafeed and cosmetics	YES		Market know-how	M73.2.0		Licensing	NOVA (owner) VFT URDV
Commercial exploitation	Evaluation of Socio-economic impacts	NO		Market know-how	M73.2.0			NOVA (owner)