Editorial Hans Reith, Project Coordinator

The potential of multiproduct algae biorefinery is underlined in a recent, comprehensive techno-economic scenario study by Ruiz et al (2016)[1]. The study indicates that production of higher value product mixes from algae as ingredients for food and for application in cosmetics/healthcare could be profitable. According to the paper, this would require scale up of algae cultivation to 100 hectare system scale, producing ca. 6,000 tons dry biomass/year and a biorefinery facility of similar capacity. The study further emphasizes the crucial importance of cost reduction of algae biomass production (for which several approaches are identified and their economic impact evaluated) and the requirement for appropriate multiproduct biorefinery strategies to achieve economic feasibility. These issues are major topics in the MIRACLES project.

This 5th Newsletter shows that significant progress is being made by the Project partners in the area of algae cultivation incl. CO_2 concentration from air, real time monitoring of target products in the biomass, harvesting and recycling of water and nutrients as well as biorefinery of the algae biomass using (among others) "green" membrane-based separation processes. Good progress has also been made in the bioprospecting program for identification and selection of new robust industrial algal strains. Value chains have been selected for the demonstration activities in the Project which are now underway. Furthermore good progress is being made in the techno-economic and sustainability assessment. With regard to product development various applications are being targeted, following an approach that focuses on establishing real consumer benefits in using microalgae derived products. All these developments are documented in more detail in this Newsletter.

[1] 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. DOI: 10.1039/c6ee01493c.

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Progress in algae cultivation and algae preconcentration

A major topic in the Miracles project is the improvement of production and harvesting of algal biomass to achieve substantial cost reduction through: (1) Development of tools and strategies to optimize the concentration of target biomolecules in the algal biomass; (2) An innovative technology for CO_2 concentration from the atmosphere; (3) A novel Photo Bioreactor concept and (4) Application of a novel membrane based technology for combined harvesting and medium recycling.

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This year was very productive regarding the algae cultivation and algae preconcentration. Our partner, Fitoplancton Marino, selected among the earlier identified indicator genes, a promising gene (LPAT4) for rapid analysis of the TAG-synthesis status in algae cultures. New experiments were initiated to detail the correlation between gene expression and TAG-synthesis, allowing verifying and quantifying the predicting character of the indicator gene. In the work on CO_2 capture from the atmosphere, Twente University, elaborated the first design for the CO_2capturing device that is being verified. The estimated production costs are 50 euro/ton CO_2. Wageningen University and University of Huelva are constructing an updated design of the liquid foam-bed reactor, aiming at (1) providing proof of principle, and (2) longer term operation. New surfactants are being evaluated to further optimize the process. Vito and Thomas More continued their activities related to medium recirculation. Lab scale water recirculation tests with *Nannochloropsis gaditana* and *Scenedesmus obliquus* revealed that 75-90% water recirculation is possible without negative impacts on algae growth when suitable doses of N and P are added. The integrated algae harvesting & water recycling unit, based on VITO´s IPC® submerged membranes has been up scaled and improved for performing medium recirculation tests at pilot scale in 300L & 1500L closed photobioreactors in the SUNBUILT facilities of VITO & Thomas More.

The successful bioprospecting program identifies highly diverse candidate strains with special strain properties

The rational sampling approach and screening pipeline established by the partners have successfully identified robust strains with high productivities and industrial potential. The main focus will be on shifting towards specific characterization and functional evaluation of selected strains in collaboration with the industry partners in the MIRACLES consortium.

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The joint bioprospecting campaigns by the WP2 partners in Norway, Spain and Chile, has contributed to the screening of almost 40 000 isolates sampled from a broad spectrum of extreme microalgae habitats in highly diverse climatological conditions (Arctic waters, Nordic fjords, sub-tropical islands, altiplanic desert and hot springs). Our rational sampling approach is taking into consideration how evolutionary pressure may have developed special strain

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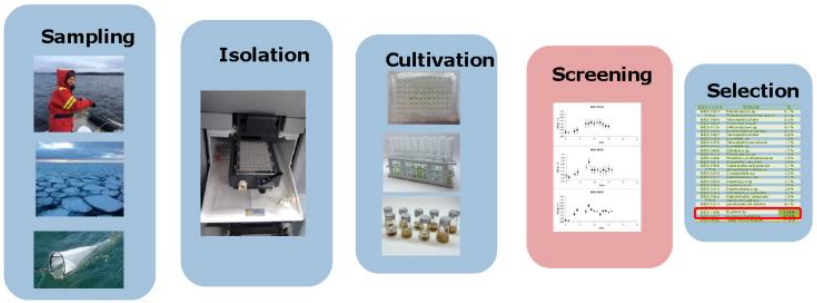
properties needed for survival in extreme habitats. The sampling design is based on the input from our industry partners in WP4 who have provided their specific requests for compounds and functionalities with commercial potential. After the initial screening to promote the cultivation of robust and productive strains, a total of 255 clonal isolates have been established and incorporated in strain collections.



	Environmental samples collected	Enrichments cultures in lab	Individual clonal cells plated (FACS)	Clonal isolates established for cultivation	Screened strains	Candidate strains
FCPCT, Spain	130	260	8640	40	10	9
UA, Chile	69	360	19200	66	20	13
UniRes/UoB, Norway	55	100	10560	149	40	5
Total	**254**	**720**	**38 400**	**255**	**70**	**27**

While the sampling and initial screening efforts are still ongoing, the focus of WP2 has now moved more towards cultivation experiments and the systematic evaluation of the industrial potential of these novel isolates. So far, the rationale of our sampling strategy has been successful, leading to high diversity and interesting strain properties, and the established screening pipeline has proven fit for purpose to identify a total of 27 candidate strains that match the industrial “wish-list” and pre-defined selection criteria. The current list of candidate strains covers the whole range of market application areas that has been high-lighted by industry partner: algae ingredients in food, aquafeed, biobased materials, and specialty compounds. In the following period, the selected strains will be further characterized, assessed for food safety, and functionally evaluated in collaboration with WP3 and WP4 partners, while we will continue to push more novel isolates through the screening pipeline in order to increase the number of candidate strains.

A major part of WP2 is toestablishidentical outdoor photobioreactor systems at each partner location, which will represent a unique possibility to compare hard productivity data of reference strains in identical reactor systems under different climatic conditions. The installed GWP-III reactor systems have been fully operational at the University of Bergen for a year, where local champion strains are currently being explored and compared with industrial strains. While the identical system is already installed at the University of Las Palmas, technical and bureaucratic issues have delayed the initial experiments in Gran Canarias and also in Chile. However, efforts are being made to have all three systems in operation during 2016, and use these for inter-comparison to monitor how the different climates and varying environmental conditions affect the growth, biochemical profiles and commercial potential of standard microalgae strains. This will contribute to assess the cultivation in areas with limited potential

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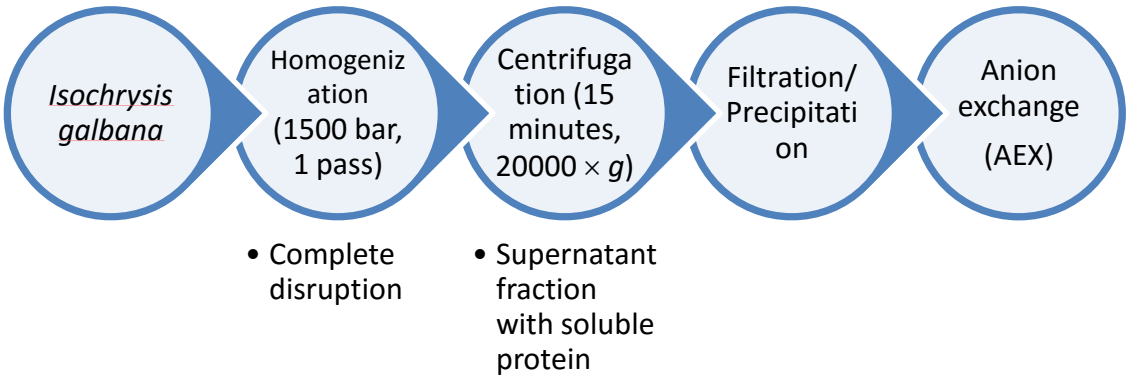
for agriculture, and contribute to broaden the resource base and product portfolio of the algae industry.

In addition to the tasks on bioprospecting and outdoor cultivation, WP2 partners at the University of Wageningen and FitoPlancton Marino are collaborating to understand the organization and regulation of the lipid pathways in *Nannochloropsis gaditana* to enable improved EPA and TAG production. Combined efforts are made to optimize productivity through metabolic modeling by an experimental approach studying the fatty acid metabolism of *N.gaditana* using 13C-labelling during N-starvation. In parallel, differential expression data from outdoor production cycles will improve the metabolic model, and contribute to a better understanding of how the metabolic flux can be optimized by either monitoring the cultivation conditions by early markers, tightly controlling the cultivation conditions by adjusting N-levels, or by employing strategies based on metabolic engineering.

"Green" separation processes

"Wageningen Food & Biobased Research" made good progress in separation of interesting components. For protein isolation, the scheme below was used. Ammonium sulfate precipitation appeared a feasible technology to precipitate (and later re-solubilise) protein. Improvement of recovery efficiency and purity is currently under study. Furthermore, techno-functional properties such as solubility and emulsification, foaming and surfactant properties will be established.



By using ultra filtration, color components were very well retrieved in the retentate. At higher protein concentrations, however, the recovery of proteins in the filtrate was lower than expected which may be due to the strong covalent linkages between proteins and other components. Consequently, the process requires optimization in order to improve the filtration efficiency and the yield of proteins obtained in the filtrate.

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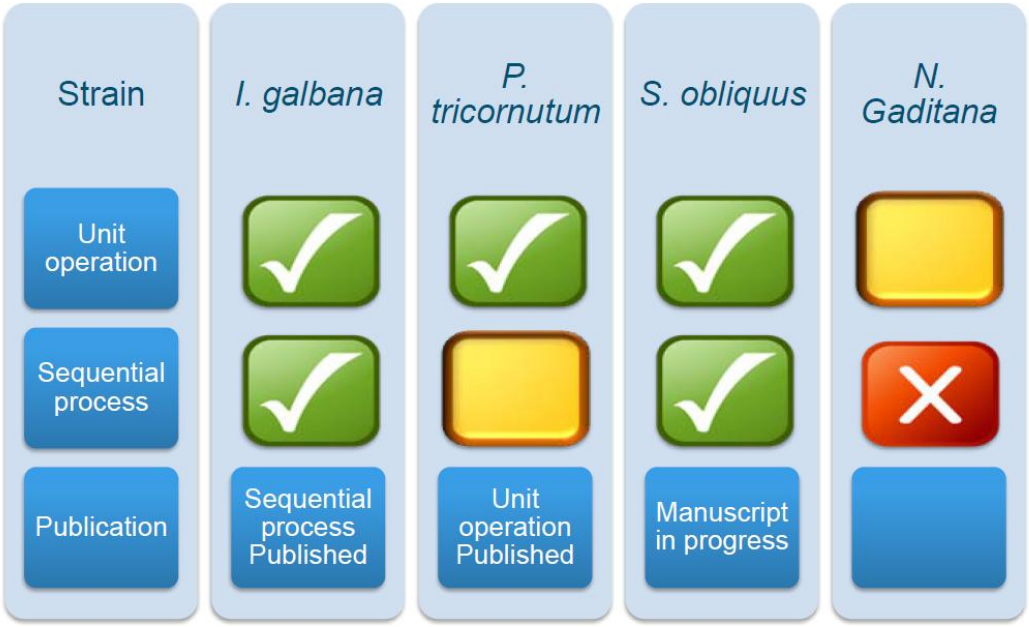
Partner CSIC completed and published several studies in algae biorefinery on *Isochrysis galbana* and *Phaeodactylum tricornutum*. They succeeded in the downstream processing of freeze-dried *I. galbana* [Green Chemistry, 2015, 17, 4599-4609], starting from non-polar and ending with polar solvents and develop unit operations for*P. tricornutum* (Food Res. Int., doi:10.1016/j.foodres.2016.04.022)



Consumer benefits in Aquafeed

WP4 evaluates the potential of microalgae in a broad range of applications, from aquafeed, feed, functional supplements to materials. In all fields, there is significant progress. However, for this newsletter, we chose to highlight one application, aquafeed, and more specifically the first results obtained by our partner SPAROS. It illustrates the novel approach followed by MIRACLES, looking more for real consumer benefits in using microalgae.

Current microalgae biomasses are not cost-competitive as bulk feed ingredients. However, the future presents new consumer-driven challenges that are shifting the focus away from cost minimization production strategies, towards enhanced consumer confidence and product value. Therefore, at the moment, microalgae biomasses may have a great potential in the animal feed sector as a functional ingredient (products incorporated in the feed to convey a benefit above and beyond fulfilling the basic nutritional needs). The feed industry is constantly seeking innovative nutritional ingredients that could add value (nutritional or other) to their traditional feeds during sensitive periods of the production cycle or specific situations resulting from farming practices.

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Since May 2016, SPAROS and FITOPLANCTON have initiated the experimental activities for demonstrating the efficacy of several microalgae as functional ingredients in aquaculture fish. The algae chosen for testing are *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*, incorporated in feeds either as whole-cells or disrupted.
Senegalese sole (*Solea senegalensis*), an emerging high-value flatfish species and gilthead seabream (*Sparus aurata*), the major produced species in the Mediterranean region were chosen as target species.

The functional role of the microalgae is being assessed at various sensitive stages of fish development. At larval and juvenile stages, the potential benefits of algae are assessed not only in terms of growth performance, but also on enhancing stress resistance and lowering the incidence of skeletal malformations. Additionally, diets with *Phaeodactylum* biomass will be fed to commercial size seabream to evaluate the effect of this fucoxanthin-rich alga on reducing body fat accumulation, natural skin pigmentation patterns and organoleptic traits.

Selection of Value Chains for demonstration

The partners from WP5 have selected 5 interesting value chains in M24 ahead of the scheduled time (M30-M36). Relevant process steps for these chains were developed. These 5 value chains are: Whole cell, Oil & Lipids, Proteins & Carbohydrates, Food Specialties and Non-Food Specialties. The work package includes pilot scale production of algae batches with optimized composition and validation of selected processes and application testing at pilot scale. The demonstration work takes place in M36-M46, although we have some activities running by M30. We have focused on pilot production batches where cultivation is optimized to obtain better oil composition based on product specifications given by industrial partners. We started validating the operation in 700 L PBR's and a complete metabolomic and transcriptomic analysis was performed. The initial results have been successful and we have scaled up to 4.000 L PBR's in order to obtain sufficient biomass for demonstration work by industrial partners. This scale up process is ongoing.

Preliminary tests have been performed for proteins but not at pilot scale yet. We have produced a batch of biomass to be applied in aquafeeds. Evaluation of the efficacy will be undertaken via pilot scale feeding trials in the coming period.

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Evaluation of selected integral value chains and life cycle assessment of microalgae cultivation

Aiming at the implementation of a techno-economic and sustainability assessment of a multi-product integrated biorefinery for microalgae.

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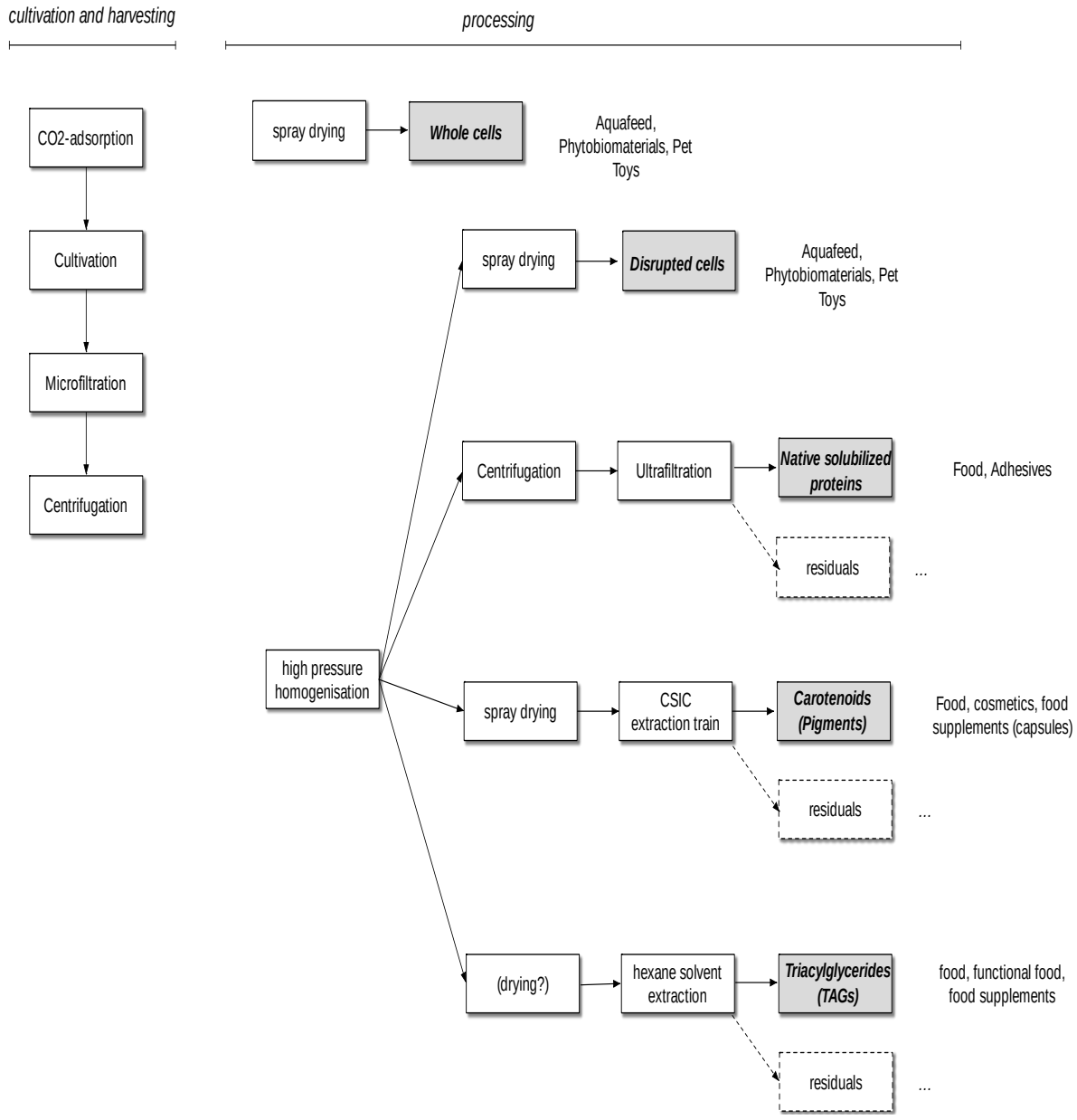
Being half-way through the project, the definition of specific value chains and scenarios is the major aim in this phase. Huge progress was made in defining the exact routes of the downstream processing and to identify hot spots in the innovative process technologies. Five value chains were defined for the techno-economic evaluation and the LCA aiming at production of (1) whole cells, (2) broken cells, (3) soluble proteins, (4) pigments and (5) TAGs.



Innovative value chains and related applications in the MIRACLES project

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The calculation of mass and energy balances is ongoing. Lab-scale results from the project are translated to large-scale by engineering rules Where applicable, scenarios such as avoiding a drying step (paste) or considering the production of stressed biomass will be investigated to first: evaluate cost aspects and second: to reduce the environmental impact by saving energy for the drying process. First results indicate that potential savings within the process chains are very promising and impacts from a life cycle and cost perspective will be further investigated.

The shift from a horizontal tubular PBR to an innovative cultivation system promises further reductions of costs and environmental impacts and will be included in the model in the coming months. Based on the process integration, cost calculations and life cycle assessment has started. First results will be available by the end of the year.

Additionally, a study to assess the consumer acceptance of algae biomass for different applications is in preparation. The goal is to identify the image as well as USPs for algae-based products to support the positioning of algae in the market.

3rd Intermediate Progress Meeting held in Bergen, Norway, on 18th, 19th and 20th May, 2016

The project's 3rd Intermediate Progress Meeting took place in Bergen, Norway bringing together all consortium partners for three days from 18 to 20 May 2016. The meeting was organized by local partners University of Bergen, Uni Research and partner ID Consortium. The meeting focused on a review of the progress achieved to date. R&D results were presented and discussed by the partners. Specific attention was given to the status and further development of process chains, identification of bottlenecks and requirements for exchange, feedback etc. from all partners working on the production chain.

The meeting included a visit to the University of Bergen labs and facilities including the pilot scale flat panel photo bioreactor operating in the framework of the project. On the 20th May the meeting concluded with a fruitful visit to the CO_2 Technology Centre Mongstad (TCM) and National Algae pilot Mongstad. More information can be found on our **Facebook** page.

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