Editorial Hans Reith, Project Coordinator

The MIRACLES project addresses major hurdles for expansion of the algae industry by enhancing cost-effectiveness of algae production and processing through innovation, development of multiple-product biorefinery technology for specialties from algae and development of new products for food, aquaculture and non-food.

In this way the project will contribute to bridging the gap to achieve industrial scale production of microalgae and application of derived products through cost reduction, establishing and validating technical and economic data and the development of validated business plans.

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

Good progress is being made as can be seen from the case studies and other updates presented in this 6th Newsletter.

Project website

http://miraclesproject.eu/

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Case: Integrated algae pre-harvesting & water recycling device

Microalgae are considered to have high potential for a biobased economy. Recirculation of water (medium) during algae cultivation and new approaches for algae harvesting could help to improve the economics of the cultivation process. Due to the low concentration of the algae in the cultivation medium (ca. 0,2 g/l dry algae in open ponds and 2 g/l in photobioreactors), large amounts of water need to be processed to produce algae paste. During algal biomass cultivation, the harvesting step (i.e., separation of single cell algae from the growth medium) is a considerable part of the capital and operational cost. These processes typically require high amounts of chemicals and/or energy.
Within Work Package 1 (WP1) of MIRACLES project, an **integrated submerged membrane based algae pre-concentration and water recirculation system** was developed and examined by VITO for harvesting of *Nannochloropsis gaditana*.

In a first stage, the performance of this Membrane based Algae Filtration (MAF) technology was evaluated at lab scale (0.1 m²/membrane) using different membranes types. Growth medium with algae biomass (0.8-2 g/L DW) was filtered via a submerged membrane resulting in (1) pre-concentrated algae and (2) filtrate that can be recycled (including salts & remaining nutrients).

Next, the MAF-process was scaled-up to semi-pilot scale (1 m² membrane surface) using IPC®-membranes patented by VITO, and were operated in batch as well as continuously. In batch concentration tests, fluxes gradually decrease with increasing algae densities, but > 75% of the water can be removed with relatively high fluxes. Cell densities > 25 g/L could be reached. A multiple-step continuous approach was tested.

Finally, the MAF-process was linked to larger scale 300L/1500Lphotobioreactor (EFRO Sunbuilt infrastructure constructed by VITO & Thomas More) where IPC®-MF membranes were used for filtration of the water before re-use. During 10 subsequent recirculation cycles, about 1/3 of the 300L reactor volume was filtrated and recirculated each time (75% recirculation, 25% fresh medium). The filtration was stable and proved to be robust. Compared to a control 300L photobioreactor (no recycling of medium), it could be concluded that the water recycling did not negatively influence the algae growth. The findings are in agreement with results from lab-scale tests performed by Thomas More. These lab scale 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 NP are added.

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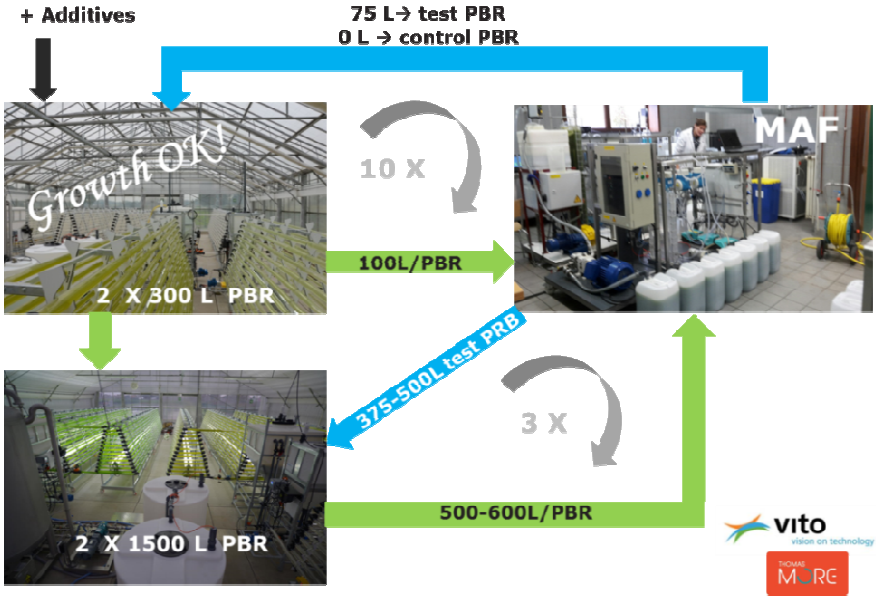

Figure 1: Water recirculation experiment using the MAF-device in the Sunbuilt pilot (Belgium)

The viability of the algae cells after the MAF-filtration step was evaluated by monitoring their growth in fresh medium. At lab and pilot scale, the viability of the algae was excellent allowing using the cells as inoculum. This proves the mild character of the MAF-technology. The MAF-technology will be leveled to a prototype towards commercial applications.

Other updates from Miracles WP1 include:

- **Development of technology for CO_2 concentration from the atmosphere.** A novel radial flow contactor was designed, constructed and tested by University of Twente for adsorption performance. Results are in line with modeling and fixed bed experiments. The results allow for the next development phase: implementing sorbent circulation and regeneration.
- **Development of a novel Liquid Foam Bed flat panel Photobioreactor concept** by Wageningen University & University of Huelva. Different reactor types were designed and tested to obtain stable foam conditions. A set-up resulting in stable algae-containing foam was obtained.

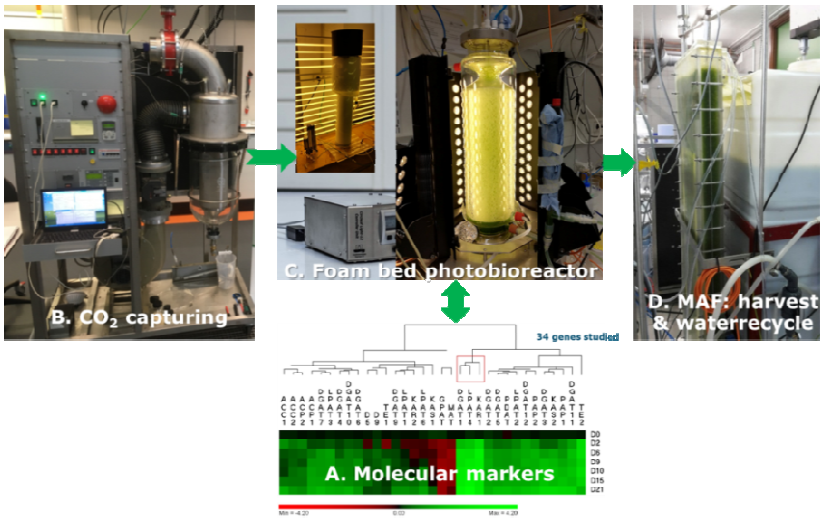

Figure 2: Technology & tools developed for improvement of the algae growth & harvesting.

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Characterization of Selected Strains and Functionality Evaluation

In this period we WP2 partners have further characterized the selected strains, and functionally evaluated in collaboration with WP3 and WP4 partners, while we also continued to push more novel isolates through the screening pipeline in order to increase the number of candidate strains. A manuscript is being prepared for publication by UoB on the strain isolation work.

As a first step for the characterization of the strains isolated by UoB/UniRes a 23 factorial design experiment was performed with a selected strain (the diatom *Attheya septentrionalis*) to determine optimal growth conditions and to find best combinations of temperature, salinity and irradiance enhancing biomass and EPA productivity. High temperatures proved deadly for this strain, which was not completely unsuspected as it was especially selected as cold-adapted strain.

Furthermore, an inter calibration experiment of the Algaemist photobioreactor amongst WP2 partners was performed by doing a repeated batch culture experiment *of Nannochloropsis gaditana*, all using the same cultivation conditions. The successful experiment revealed similar biomass yield on light and showed that results amongst partners can be compared.

In Las Palmas the screening of interesting strains under outdoor conditions was carried out using PET bottles hanging in a mesh (Figure xx, left) by partner FCPCT. The local strains that will be selected for further testing and production in the GWP III PBRs have been chosen according to their growth parameters, their robustness and the composition in the metabolites of interest.

Figure 3: Microalgae screening (left) and installation and preparation of the GWP III systems in Las Palmas (right)

Following the screening, biomass production of the best strains in small-scale outdoor photobioreactors is evaluated under extreme climatological conditions and the potential of these local environmental conditions for outdoor mass cultivation is investigated.

GWP-III outdoor bioreactors were installed at UoB in summer 2015. Operation of reactors was tested by cultivating the standard strain of the green algae *Scenedesmus* during August and September 2015. Furthermore, three different strains of the diatom *Phaeodactylum tricornutum* were grown successfully from April 27th until October 28th, as repeated batch cultures in the outdoor bioreactors. One of the strains was obtained from the partner Fitoplancton, the other two were local strains obtained from the isolation and screening experiments described before. Cultivation conditions and growth were followed daily to determine the biomass and PUFA productivities. Analysis of the data is currently ongoing and conclusions on strain performance are expected soon.

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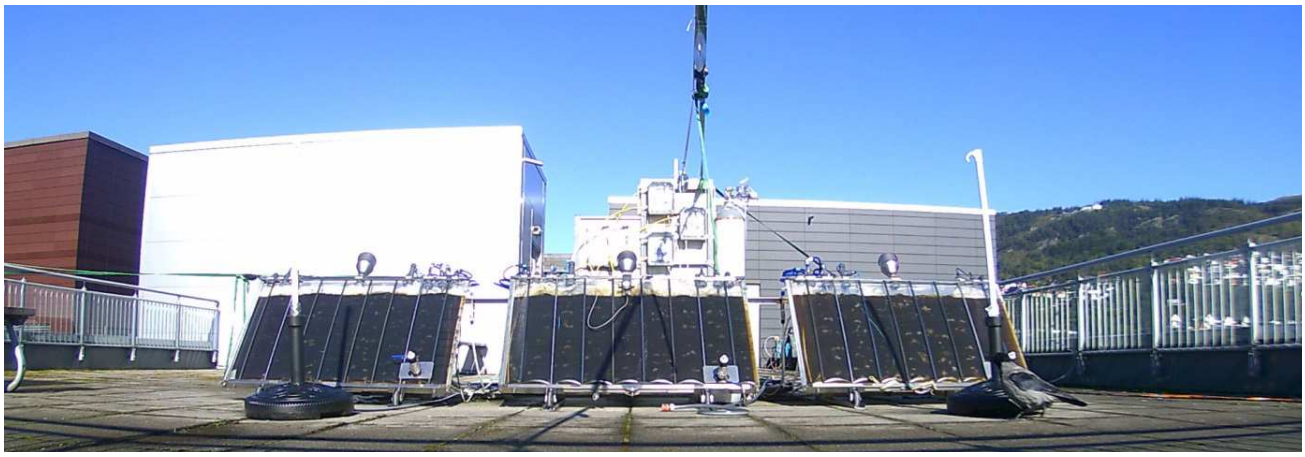

Figure 4: The GWP III systems on the roof of the UoB building in Bergen with the three *P. tricornutum* strains

In the last months the GWP III flat panel photobioreactors have been installed outdoors at the premises of FCPCT in Las Palmas (figure xx, right). Currently, the final adaptations are being made. In the meantime, incoculum is produced for all selected GWP strains, ready to start production of biomass and determine growth parameters in relation to the selected culture conditions and nutrient concentrations. A marine strain (*Sarcinochrysis marina)* and a freshwater strain (*Chlorococcumsp*.) will be compared to the control strain *Phaeodactylum tricornutum.* Moreover, biomass production in plastic bags is ongoing, to provide partners of other WPs with enough biomass for further quality assessments.

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"Green" separation processes

Wageningen Food & Biobased Research published their work on mild biorefinery processes to obtain an enriched fraction of water soluble proteins, free from chlorophyll, from the microalgae *nannochloropsis gaditana* (Bioresource Technology, Vol. 225, pp 151–158).

The results showed that for cell disruption homogenization was more efficient than a protease treatment in terms of cell disintegration and release of soluble proteins. However, the filtration process revealed a higher efficiency for the protease treated samples. The study also concluded that increasing the cutoff of the membrane does not necessarily improve the performance of the process, especially for native proteins.

With regards to the Evaluation of green sequential pressurized processes for algae biorefinery carried out by CSIC. After succeeding in the downstream processing of the marine microalgae *Isochrysis galbana* [Green Chemistry, 2015, 17, 4599-4609], starting from non-polar and ending with polar solvents, CSIC (Madrid) developed a green platform for the fresh water microalgae *Scenedesmus obliquus*.

The results and main conclusions have been detailed in a manuscript that is currently under revision, as well as in an international conference: European Roadmap Conference for an algae-based industry (held in April 2016 in Olhao, Portugal).

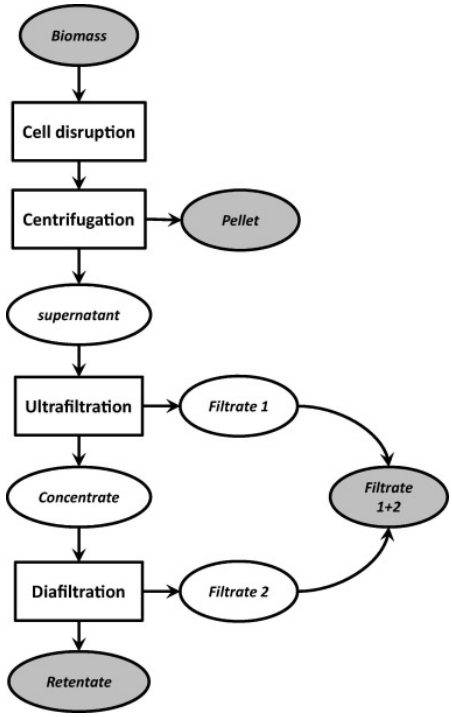
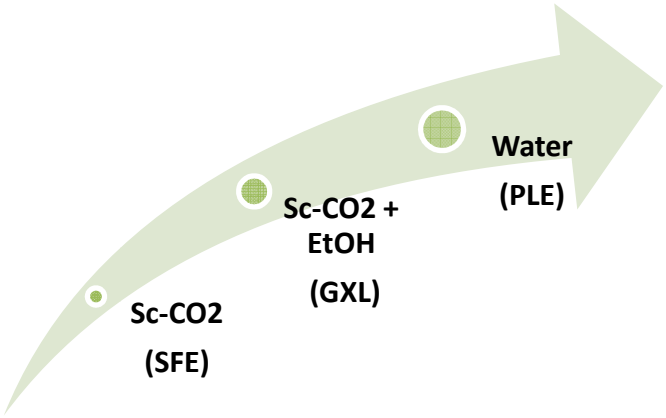


Figure 5: Schematic representation of the overall process from cell disruption to diafiltration.



On the other hand, a new approach for the selective extraction of bioactive compounds from the marine diatom *Phaeodactylum tricornutum* based on the theoretical modeling of Hansen Solubility Parameters (HSP) was presented in the Conference Alga Europe (held in December 2016 in Madrid, Spain). The results and conclusions of the study are detailed in a manuscript that is currently under revision in an international scientific journal.

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Innovation breakthrough in application world!

In the previous newsletter, we highlighted the aquafeed targets and progress in MIRACLES. Several aquafeed formulations containing microalgae are now already available: formulations for juvenile fishes, for shrimps and for ornamental fish already demonstrated an interesting potential for microalgae in products with enhanced consumer value. More trials are ongoing / scheduled for seabream, Senegalese sole and salmon. Processing-wise it is possible to formulate aquafeed by extruding directly algae slurry, eliminating the need for complete dewatering of algae biomass.

New algae specialties

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

New bulky applications

Protein-enriched algae extracts (e.g. after oil extraction) can replace up to 30% of phenol in adhesives for plywood. Similar fractions or whole cell algae are effectively incorporated in aesthetic biomaterials for flooring, table top and light covers and have been compounded with thermoplastic starch to be injection molded.

New positioning strategy

Microalgae still is a rather expensive biomass source. A lot of attention went towards developing a proper positioning strategy for microalgae products created in MIRACLES. Such a positioning strategy balances rational, emotional and intuitive arguments in order to 'sell the price' of the algae components in the different applications. Focus needs to shift from a technical message towards a more subjective message, creating a sentimental connection between consumer and product, addressing the consumer's personal values and aesthetic aspirations. Make microalgae cool!

This strategy has been proven effective in biomaterials. We are now busy implementing it to microalgae.

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Proof-of-Concept for Integrated Value Chain

The aim in MIRACLES WP5 is to demonstrate integrated value chains to deliver proof-of-concept and demonstrate techno-economic viability.

The Team already selected 5 value chains ahead of schedule in M24, although the period for this task was M30-M36. 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 on M36-M46, although we have some activities running by M30. We have focused pilot production batches where cultivation is optimized to obtain better oil composition based on product specifications given by industrial partners. Initial operation on 700 L PBR's was validated and a complete Metabolomic and transcripotomic analysis was performed.

The initial results have been successful and we have scaled up to 4.000 L PBR's in order to obtain enough biomass for demonstration work of industrial partners. This scale up process is ongoing both for aquafeeds and oil production. Preliminary tests have been performed for proteins but not at pilot scale yet and all batches of biomass for Aquafeed applications have been produced.

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

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

Recent work focused on (1) definition of scenarios for the value chains to be evaluated and (2) continued the work on conceptual process design models and the initial economic evaluation of the value chains. Scenarios and conceptual process chains (Fig. 1) were proposed based on the five product value chains selected for demonstration in WP5 for a deeper investigation (whole cells, broken cells, native soluble proteins, pigments, TAGs).

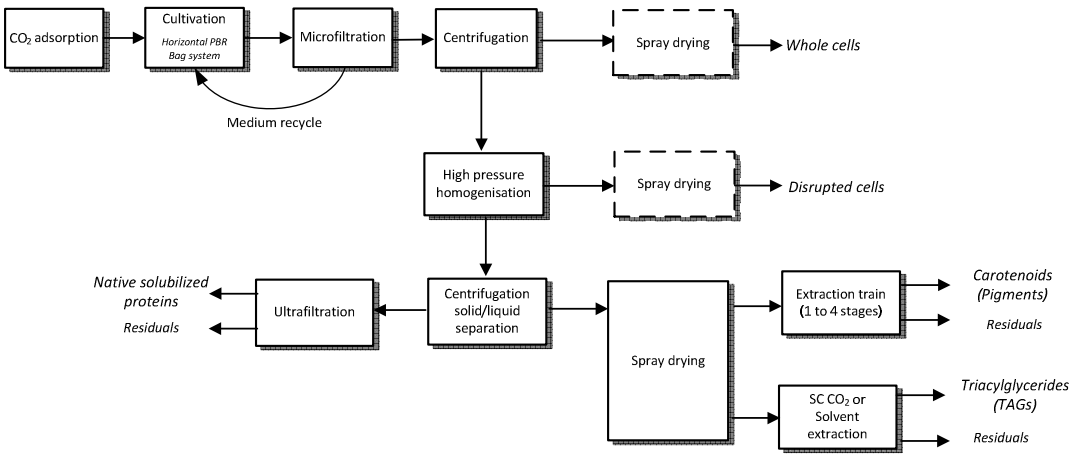

Figure 6: Overview of selected value chains.

Together with WP 3 the process integration for the 5 biorefinery chains was performed, with algae species specific data. Moreover, a first economic evaluation of the biorefinery was performed. The current cost estimates show that for most value chains the cultivation costs are a major contributor to the cost. The cultivation cost represents at least 70% of the total biomass cost, for scenarios whole and broken cells as well as the chain aiming at proteins. For the pigment value chain the cultivation costs are around 40% of the total cost. For the TAG case, the cultivation costs are two-third of the total cost. However, product costs depend strongly on the algae species, due to species specific requirements and yields.
The key conclusion is that further exploitation of the residuals remains a point of attention, to 1) improve the overall economics, 2) reduce “waste”, and 3) reduce the environmental impact per component.
The final project period will focus on generation of co-products and/or valorisation of residues to further develop the process design of the value chains that will be fine-tuned and consolidated in a joint effort of WP3, WP4, WP5 and WP6.

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

Moreover, the project will conduct a consumer study assessing potential benefits and concerns from consumer side. The goal is to identify the image as well as USPs for algae-based products to support the positioning of algae.

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Miracles 3rd Annual Progress Meeting Held in Faro 15-17 November

The Miracles Project 3rd Annual Progress Meeting was held in Faro, Portugal, hosted by project partners Sparos and IDConsortium.

The meeting took place during the 15th to 17th of November 2016 and it was a great opportunity for all project partners to share their latest updates.

The meeting concluded with a visit to the IPMA Aquaculture Pilot Station. https://www.ipma.pt/pt/pescas/eppo/index.jsp

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