

FUTURE EUROPEAN LEAGUE 4 MICROALGAL ENERGY

to develop a sustainable, scalable process
for biofuels from microalgae and to
valorize the by-products by 2017



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WHAT IS FUEL4ME?

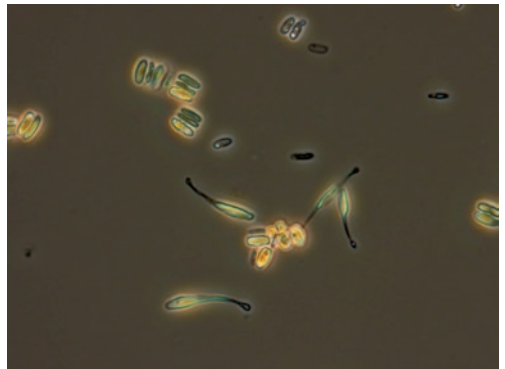
- | European Project funded by the 7th Framework Program with a budget of 5,4 M€.
- | It was developed since 1st of January, 2013 until 31st of December, 2017.
- | The project coordinator was Wageningen Food & Biobased Research.

The main Fuel4Me goals were:

- To establish a sustainable chain for continuous biofuel production and valorize the by-products using microalgae as a production platform, thereby making 2nd generation biofuels competitive alternatives to fossil fuels.
- To contribute to the transition to a sustainable energy model.

To achieve these goals, the planned objectives and activities were:

- To develop a continuous one-step process in which the lipid productivity in microalgae cultures were maximized under different growth conditions and the lipid profile was optimized for the biofuel production by researching on: genes, metabolism, biochemical aspects and bioprocess engineering.
- To translate the developed one-step process to outdoors in order to achieve a robust and reliable production process with short downtime and a continuous lipid production under different climates: FUEL4ME set up microalgae production in 3 pilot plants (Italy, Netherlands and Israel) and 1 demonstration facility in Spain.
- To develop and integrate an innovative and continuous downstream process for conversion of microalgae into biofuels with consistent volume and quality, resulting into a technically feasible and sustainable process chain.
- To demonstrate the capability of the optimised process at a pilot scale under representative industrial conditions in a pilot facility in Spain.
- To assess the environmental, social and economic sustainability of the continuous production and conversion process developed by FUEL4ME consortium.



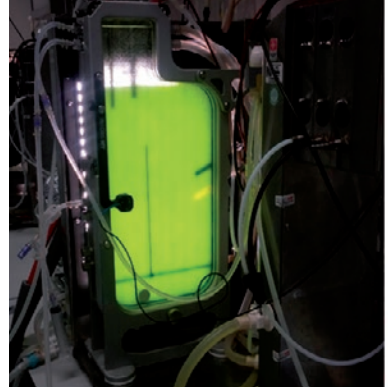
RESULTS, LESSONS LEARNT AND CHALLENGES FOR THE FUTURE:

1. Continuous vs batch production?

The Universities of Wageningen and Ben Gurion and the spin off from University of Firenze (Fotosintetica and Microbiologica s.r.l) were in charge of developing and translating to outdoors of the continuous one-step process.

The main conclusions were:

- a. FUEL4ME has demonstrated (in the lab and outdoor) the potential of a one-step lipid production process with *Nannochloropsis*, which has comparable lipid productivity to the traditional batch process.
- b. The costs of the entire process (upstream+downstream) should in the end be decisive for ultimately adopting one of the two cultivation strategies.



GWP®-III reactors at F&M pilot plant (Florence-Italy)



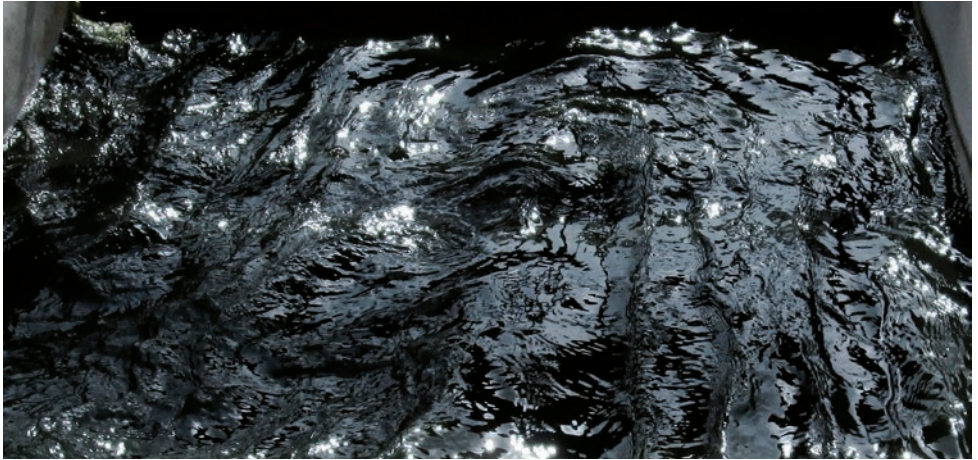
Flat panel reactor for outdoor microalgae cultivations at AlgaePARC (The Netherlands)

2. New insights into the biogenesis and regulation of lipid globules:

Globule associated proteins were isolated and identified by proteomics, and bioinformatics. One of the proteins is a novel class of LD (lipid droplet) proteins found only in diatoms. Overexpression of all four candidates fused to Green Fluorescent Protein localized to LDs under nitrogen starvation. Overexpression of a GFP fused green algae LD protein also localized to LDs and significantly enhanced TAG productivity under nitrogen starvation. In conclusion, results indicate that a significant number of LD proteins are involved in LD formation, TAG accumulation and LD translocation and fusion.

3. Thanks to Fuel4Me, Evodos improved their technology (Evodos 25) for harvesting & water recycling obtaining the following conclusions:

- a.** The successful integration of improved splash screen in fully automated harvester minimized paste losses.
- b.** The separation efficiency was higher to 90% and the harvesting capacity is higher to 6m³/day for the selected microalgae strains.
- c.** The water-recycling unit was successfully tested, however more research is needed on re-using the recycled water for cultivation (mainly in performance, need for adding nutrients etc.)



4. Cellulac have developed a technology which is ready to be commercialized for cell disruption (SoniqueFlo) which may bring significant advances in terms of costs and environmental benefits:

- a.** 25% of operational costs saved
- b.** Less capital expenditure.
- c.** High energy efficiency during the process
- d.** It is a solvent free solution
- e.** It has a very small carbon footprint.
- f.** It is ready to be commercialized

The challenge is the amount of microalgae biomass to be disrupted. The technology is designed for the treatment of large amounts of biomass. The first installation is in place and production of 127 tonnes is currently underway in the UK with further installations and production to follow in 2017.

5. Feyecon was in charge of the primary extraction of lipids with supercritical fluid with the aim of obtaining the highest energy efficiency and reduce the costs of the process. Significant advances has been achieved:

- a. Extraction of TAG with very high efficiencies (>95%)
- b. The biomass after extraction looks stable and has high value
- c. Data for scale-up was collected and realistic costs calculations are made

It is important to highlight that:

- | Microalgae biomass must be dried (wet extraction is not feasible) and it is important it is cracked in order to obtain high yield
- | Physical properties of dried and cracked biomass has significant effect on extraction yield.
- | Modifier is not a good option for extraction of FAs from microalgae as it is not selective
- | Extracted oil needs polishing (drying, FFA removal, deodorization, bleaching, dewaxing)



Feyecon was also in charge of the economically feasible provision of high value and low value fatty acids. They have developed novel reactive extraction and separation protocols based on advanced supercritical fluid technology (instead of chromatographic methods). The following results were obtained:

- d. PUFAs were purified >81% which is higher than other technologies
- e. Concentration of PUFAs was stable and robust during 60 hours (in continuous running).
- f. FFAs content of oil was reduced from 23% to 1% at 50C (promising for today's vegetable oil main challenge).

It is important to highlight that:

- | Oil must be pre-treated before fractionation (de-waxing, solid removal and bleaching).
- | PUFAs in esterified form is purified better at low pressures (110-150bars).
- | By-product of fractionated PUFAs is already biodiesel.
- | Separation of FFA from TAG is possible at low temperature.

6. Neste analysed three algae oil samples from Feyecon for suitability to Neste renewable diesel

- a. All samples were suitable for NEXBTL process in terms of impurities.
- b. However chlorine levels were rather high and material suitability should be checked. High chlorine levels increase requirements for materials. The oil is usable for hydrotreatment if the materials of plant are chlorine resistant.

It is important to highlight that:

- Neste recommends that PUFAs and unsaturated fatty acids should be removed before hydrotreatment.



7. Neste hydrotreated algae oil in microreactor to Neste renewable diesel

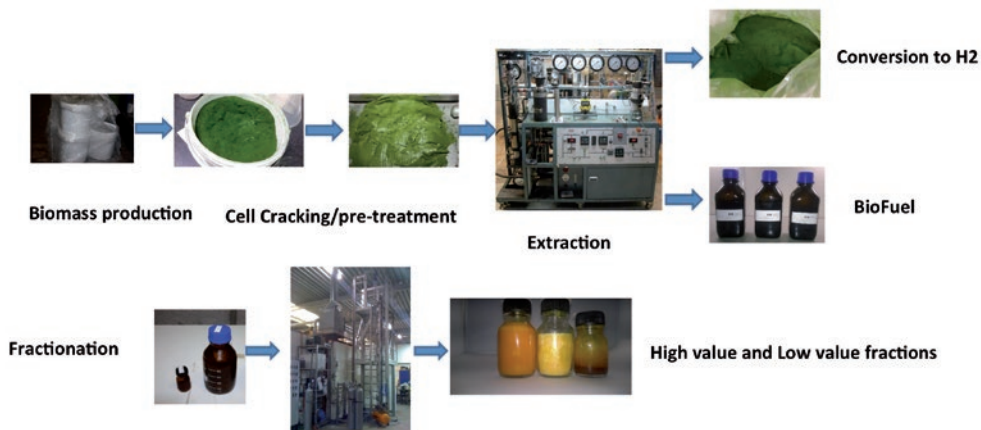
- | Testing could be performed in typical process conditions successfully.
- | Pre-treated algae oil was tested in microreactor-scale at Neste to produce NEXBTL products. The microreactor testing simulates the process conditions in existing commercial NEXBTL-plants.

It is important to highlight that:

- | Testing was done with with blend of 20 % algae oil with 80 % of regular NEXBTL feedstock. Blending ratio was determined by chlorine content of the sample.
- | Testing could be performed in typical process conditions successfully.
- | High amount of PUFAs can lead to oligomerization and heavy compound formation. Neste recommends oil fractionation before hydrotreatment.
- | Accumulation of chlorine in the oil should be avoided during cultivation.
- | Neste renewable diesel is a premium-quality drop in fuel compatible with all diesel engines and it is already commercially available.

Demonstrate optimized process at pilot scale

(Feyecon, Proviron, Evodos, Cellulac, NOIL)



8. Proviron together with Norsker Investigaciones S.L were in charge of setting up and operating the demonstration plant sited in Huelva (South of Spain):

■ A demonstration plant of 264 m² (22 reactors) was set up in Huelva using the ProviAPT disposable flat panel photobioreactors. One of the advantages of the system is the heat conservation due to the large water volume of the reactors. This was demonstrated during the project: a heating/cooling energy requirement of only 1.5 kW per reactor during the two hottest summer months – coldest winter months, respectively.

The biomass productivity was satisfactory: 24 % lower than computer model projections suggested for given temperature and light conditions, in a whole-year projection, close to 800 kg of dry weight.

Proviron and Norsker Invesatigaciones have furthermore gained valuable experience with material stability under south European conditions and hygienic plant design and process development, which will be applied in the further development of outdoor microalgal cultivation.

■ It is important to highlight that the results from FUEL4ME demonstration plant have shown the potential to continue doing research with a focus on productivity and solving some gaps related to contamination (prevention and control) problems.



9. The main conclusions from the sustainability assessment made by Joanneum were:

- A full-scale integrated micro algal-based process with sustainability indicators of the whole value chain in comparison to a conventional reference system including the three dimension economic, environmental and social aspects was modelled
- A comparison of the modeled full-scale integrated micro algal-based process with the assessment of to actual state of technology shows information on key critical parameters impacting the sustainability of the FUEL4ME integrated process.
- Main influences on the sustainability: cultivation and harvesting, electricity demand, source of CO₂, source of water, suitable land.
- Due to the current state of the art and the big amounts needed for biofuels it seems that microalgae cultivation for biofuels appears less feasible than for high value products, e.g. PUFA. Further technology development needed to improve economic and environmental sustainability. FUEL4ME believes that a long term innovation strategy, first with a stronger focus on higher value products, will result in economically feasible and environmentally sustainable microalgae-based products.





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