PROJECT FINAL REPORT

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Final publishable summary report

1. Executive Summary

The targets of the PUFAChain project were highly purified omega-3 fatty acids, i.e. Poly Unsaturated Fatty Acids (PUFAs). The Docosahexaenoic (DHA) and Eicosapentaenoic (EPA) acids are of particular interest for nutrition and pharmaceutical applications. Microalgae were chosen as an alternative and sustainable source. Because the traditional source of PUFA production, fish oil, is an ever decreasing resource associated with upcoming shortages of environmental threats such as pollution of oceans as well as political worries in developing countries, the search for alternatives concerning a sustainable PUFA supply is renewed. Photoautotrophic algae are the only form of life which can readily produce and accumulated PUFAs directly using energy from the sun, the use of industrial produced microalgae is seen as a promising alternative.

The project aimed at the development and pilot-scale implementation of a complete microalga-based process from screening to feedstock production, from harvesting to oil extraction and purification, from laboratory to prototype scale. A sustainability assessment based on real data from practical experiences from each step of the chain was performed. A consortium of six companies and three research institutes combined research and industrial process development, building the links of the value chain.

The value chain started with the screening and selection of productive strains from the two culture collections, SAG and CCCryo. After more than 160 strains were tested for their EPA and DHA qualitative content, a selection of six strains were further used for upscaling. The selected strains included mesophilic and cryophilic strains to support the Algae-Crop-Rotation principle which will allow for the whole year production of PUFAs. Previously unexplored (*Prorocentrum, Chloridella, Raphidonema*) and bench-mark strains (*Thalassiosira*) were further characterized and cultivation strategies were developed for both, laboratory- and industrial-scale applications, at partner A4F. After optimizations regarding the cultivation medium, pH values, CO_2 supply, disposal of O_2 including Multiy- and Uni-Layer Horizontal photobioreactors, the productivity of the novel strains could reach one of the benchmark strains. Also the cryophilic *Raphidonema* strain was successfully cultivated in an outdoor photobioreactor. For harvesting a new integrated membrane-based filtration including the reuse of process water was successfully tested on the pilot scale. This system allowed simultaneous salt content reduction and cell disruption via osmotic shock for the brackish water and marine algae strains using diafiltration. Cell disruption is an important step before extraction of the crude algal oil. Osmotic shock lead to >95% success with *Prorocentrum* and *Thalassiosira* and sonification to 70% in *Raphidonema*. For *Chloridella* a bead milling was to be the most appropriate method. For the oil extraction two technologies were tested, e.g. supercritical CO_2 ($scCO_2$) for the dried algal biomass and liquid propane for the wet algal biomass. Success of the $scCO_2$ extraction depends on that the target PUFAs were available as neutral lipids, the cell disruption was successful and the particle size after spray-drying appropriate. Although propane extraction could be tested only on a limited amount of algal biomass, the technology showed promising results. The crude algae oil composition was comparable between the two extraction methods, i.e. it had 30 – 70% lipids with the rest being unsaponifiable materials (chlorophylls, phytosterols, etc.). Unexpectedly high proportion of the free Fatty Acids were observed in crude algae oil extracts from both extraction methods, that influenced oil purification and concentration process design.Crude algae oil purification and concentration process design initially was focused on the Tri-Glycerides (TAG´s) extracted from algal biomass. The experiments with a model oil (fish-oil) showed the potential of the new enzymatic splitting of PUFA oils combined with the Short-Path-Distillation (SPD). High quantities of the free fatty acids (up to 50%) in the crude algae oil extract lead to the development of a new process strategy for the DHA and EPA producing *Prorocentrum*. All remaining and distilled-off free fatty acids were purified using a urea complexation method. An enrichment of the PUFA fatty acids (i.e. EPA & DHA) of >92% was achieved in only one purification step with an overall yield of >80 %. The integrated value chain was also critically evaluated for its environmental and socio-economic sustainability to support a commercial scale-up with lowest possible impacts.

The results show that only combination of the appropriate productive strains, targeted cultivation strategy, optimized state-of-the art harvesting, cell disruption and crude oil extraction technologies will lead to a sustainable production of PUFAs by a value chain based on photoautotrophic microalgae.

2. Project Context and Objectives

The targets of PUFAChain are high-value products from algae, in particular high purified omega-3 fatty acids, for nutrition and pharmaceutical applications. The concept of the project is to develop a value chain, i.e. to assemble a complete microalga based process from feedstock production and harvesting to oil extraction and purification, from lab to prototype - so that a new and sustainable resource of omega-3 fatty acids as well as a commercial scale-up can be further developed. A consortium with six companies and three research institutes will evaluate and develop innovative technologies by taking advantage of a complimentary partnership.

Omega-3 fatty acids, i.e. Poly Unsaturated Fatty Acids (PUFAs), in particular Docosahexaenoic (DHA) and Eicosapentaenoic (EPA) acid, are recognised as important players supporting human health. They play beneficial roles in the prevention and/or treatment of coronary heart diseases, cancer, and diabetes or occupy a structural role in the nervous tissues of the brain and retina. PUFAs are present in large amounts in fish oil and cephalopods, but the concentration of EPA/DHA in fish oil varies considerably, depending on location, annual season and availability of phytoplankton. In addition, with the upcoming shortages due to environmental threats such as pollution of oceans microalgae represent a promising alternative source for EPA and/or DHA. Through the algal cultivation process, contaminants (e.g. heavy metals) and other unwanted by-products can be avoided. Certain algal strains provide different acids much more selectively and this facilitates the further isolation and purification of target products. Remarkably, algae are the only form of life which can readily produce PUFAs directly using the energy from the sun. PUFA accumulation in algae is a response to stress, i.e. to protect the algal cells against photodamage and photooxidative injuries.

PUFAChain picks up a specific application with high market relevance, the use of highly purified omega-3 fatty acids, together with side stream products following the biorefinery concept. Within the project, two renowned bio resources for microalgae (partners UGOE and Fraunhofer with their algal culture collections, acronyms SAG and CCCryo) provide a pre-selection of the enormous biodiversity of microalgae for testing and to further explore culture techniques to optimise PUFA yield (WPs 2 and 3). For the mass production algae with optima and tolerances towards high temperatures and light intensities, best suited for the cultivation in summer in Middle Europe, will rotate with cryophilic or cryotolerant strains isolated from Polar Regions and adapted to low light and temperatures, which are well suited for growth in colder seasons. The most suitable cultivation strategies for both laboratory-scale and industrial-scale application will be developed (WP 4). The optimal processing data corresponding to the needs of the specific algae (e.g. temperatures and pH values, CO2 supply, disposal of O2, distribution of light intensity in the photobioreactor and photosynthetic efficiency) have to be investigated systematically and carefully evaluated to increase the product yield of an algal strain. For example, high oil content can be induced by N-deprivation. For optimisation of the algal biomass flat panel and tubular photobioreactors will be used for the up scaling from laboratory production tests (e.g. 10 L) to pilot scale cultivation (up to 1 m3, WP4 with partner A4F). For harvesting, new integrated membrane-based filtration including the reuse of process water will be used for algae harvesting (WP 4, partner MAHLE)). Various extraction procedures will be evaluated to reflect the sensitivity of the unsaturated fatty acids, i.e. for optimally producing high quality oils at lower costs (WP 6, partner NATEX). These include extraction using supercritical CO2 after pelletizing the algae in culture to fine powder or extraction of concentrated wet algae biomass by propane in order to provide clear oil and defatted algae pellets. The latter may be further used as animal feed in aqua cultures. Also, various novel methods for cell disruption to optimally protect the sensitive omega-3 fatty acids will be evaluated (WP 6). The crude algal oil will be purified to gain highly purified and concentrated (> 98% pure) fatty acids employing a cascade of purification steps which include organic solvent extraction, fractionated crystallisation and catalysed hydrolysis. The value chains' processes will also be critically evaluated for their sustainability, so that a commercial scale-up can be further developed.

Project objectives

- **Strain selection**. The selection of the productive strain was focused on the high EPA and DHA content as well as growth characteristics. Several strains will be evaluated to find the most suitable 2 to 5 algal strains with optimized fatty acid/lipid yields along with the growth demands maximising fatty acid and more

specifically DHA/EPA production. The exact genetic and taxonomic identification of the strain and the maintenance of a project algal strain database and provision will provide a genetically stable stock through cryopreservation. A special idea behind the involvement of the two culture collections in the project is the "algal crop rotation principle" for algal mass production. This principle means that not a single alga is cultivated during the whole year, but instead two or even more different strains varying in their temperature and light optimum for growth in different seasons of the year and the season's prevailing climate conditions. The production gains of this system as well as the economic effect will be determined. Metabolite yield of pre-selected algal strains from two life algal collections will be optimized and aligned with end-user specifications.

- **Bioprocess engineering**: The product yield from the cultured algal cells can be dramatically increased by a skilful cultivation strategy corresponding to the needs of the specific algae. To get a rational basis for this strategy the optimal processing data have to be investigated systematically. Examples of processing data are: kinetic data, time period for cultivation of cell biomass time period for forming fatty acids/lipids in the cells production (high oil content can be induced by N-deprivation), recipes for cultivation medium during the different stages of the production process, temperatures and pH values, CO2 supply, disposal of O2, distribution of light intensity in the photobioreactor and photosynthetic efficiency. In conclusion the objective of the bioprocess engineers is to develop the most suitable cultivation strategy for both laboratory-scale and industrial-scale application. Strain potential, using a new combination of existing methodologies to evaluate scale-up potential.
- **Downstream processing**: The objective of the downstream processing stage is to find the adapted viable and efficient treatment of the culture broth to get high purified omega 3 fatty acids. Therefore several treatment steps will be combined in a suitable process chain. The sub stages "Cell Disruption", "Crude Oil Extraction" and "Separation of defined molecule classes" will be investigated. Also supercritical CO2 extraction and solvent extraction will be studied. Downstream processing and water management will be developed in order to supply extracts for the formulation of new oleochemical products and save water and nutrients. A further aim is to develop an integrated membrane filtration for harvesting. The advantage of membrane filtration is the possibility to remove bacterial loads and cell fragments. The aim of this approach is an efficient water recycling and reuse of the nutrients.
- **Integrated cultivation and processing**: combining all technical steps for demonstration. A comprehensive and holistic sustainability approach, addressing environmental, economic and social aspects, will complement the scientific and commercial advances on each value-adding stage. - Industrial biomass production including harvesting and water treatment: The objective is to install an integrated cultivation process adapted to the needs of the end-users specification and the selected strains including the water management and the reuse of nutrients.
- **Product formulation**: The objective of the last step of the loop follows the aim of the first step. The aim is to develop the suitable preparation of the high-purified algae oils as well as the exploitation of the side products and residues. DHA and EPA are widely used in high value products for nutrition and pharmaceutical applications. The end-user applications will define specifications that propagate backwards along the various value-adding stages of the value chain. The different stages include biology, cultivation technology and downstream technology. Therefore the aims of this project are to realize a concrete exemplary value chain, to develop the technical interfaces between the different value adding stages and to investigate the still open research aspects on every single stage while addressing the needs of the value chain as a whole. The project is strongly industry driven. Application: The objective of the end-user is to develop a sustainable source of high-purified o-3 FA (DHA / EPA) as building blocks in modern oleo chemistry to gain high value products for nutrition and pharmaceutical applications.

Structure of the project

(1) The concept of the algae biorefinery will be picked up and put into effect by the realisation of an exemplary value chain (Fig. 1). The focus on high value-added products will be realized by the development of high-purified omega 3 fatty acids (DHA/EPA) as building blocks in modern oleo chemistry. The potential integration with other processes will be considered, e.g. by considering exploitation of accompanying high-value substances e.g. colorant antioxidants as sun protection of the skin, considering exploitation of proteins and enzymes for pharmaceuticals and polysaccharides as surface active compounds, considering exploitation of

still remaining residues in the products of a pet food producer, partner in the German network algae, and/or in a biogas plant in the neighborhood of the coordinators site.

(2) The valorisation of all products including the side streams will be ensured by the involvement of the end user IOI Oleo and its technical and economical specifications. IOI Oleo is already established in the market with similar products made from other bio resources, so the market access is already given. The economic, environmental and social viability will be monitored and evaluated by a dedicated approach conducted by two independent institutes: IFEU and DLO.

(3) The necessary industrial leadership will be carried out by the main industrial partner and end user IOI Oleo active in the oleo chemistry ensuring the future market access. This project will include the development of suitable algal strains and cultivation parameters by three relevant research institutes (SAG, IBMT) providing profound expertise in microalgae.

(4) The project can build up on already performed scientific work relevant to the end users' needs in particular concerning the fatty acid profiles of microalgae. Since the target end-user markets do not allow genetically modified organisms the work will restrict to biodiversity exploration, bioprospecting and the investigation of natural growing conditions. The improvement of photosynthetic efficiency, the customizing and maximizing added value products yields and the development of algae cultivation methods will be done the bioprocess engineering partner A4F. The design and development of different cultivation systems with innovative and efficient configurations will build on the long-standing experiences of the industrial provider of algae and algae cultivation technology A4F.

(5) The harvesting and water management will be done by A4F (long-standing experience in algae cultivation) and Mahle InnoWa (long-standing experience in water management and newcomer in the algae community). Mahle InnoWa will develop a new integrated membrane filtration and the reuse of process water.

(6) The suitable downstream processes will be developed in cooperation of two partners: NATEX will investigate supercritical and liquid solvent extractions. Cremer will examine several process steps concerning cell disruption. Product extraction, purification and formulation will be done by the end-user Cremer using partly already existing equipment formerly dedicated for other purposes. To demonstrate the whole process a demonstration plant will be built at the end-users site combining all necessary steps and demonstrating the viability of all technical interfaces.

(7) The overall economic, social and environmental sustainability will be monitored and evaluated by the two independent institutes IFEU and DLO. These institutes will not only work retrospectively observing technical developments but will also actively provide other participants with advice during the realization of the project. Through such an iterative approach the early implementation of economic, social and environmental needs will be ensured.

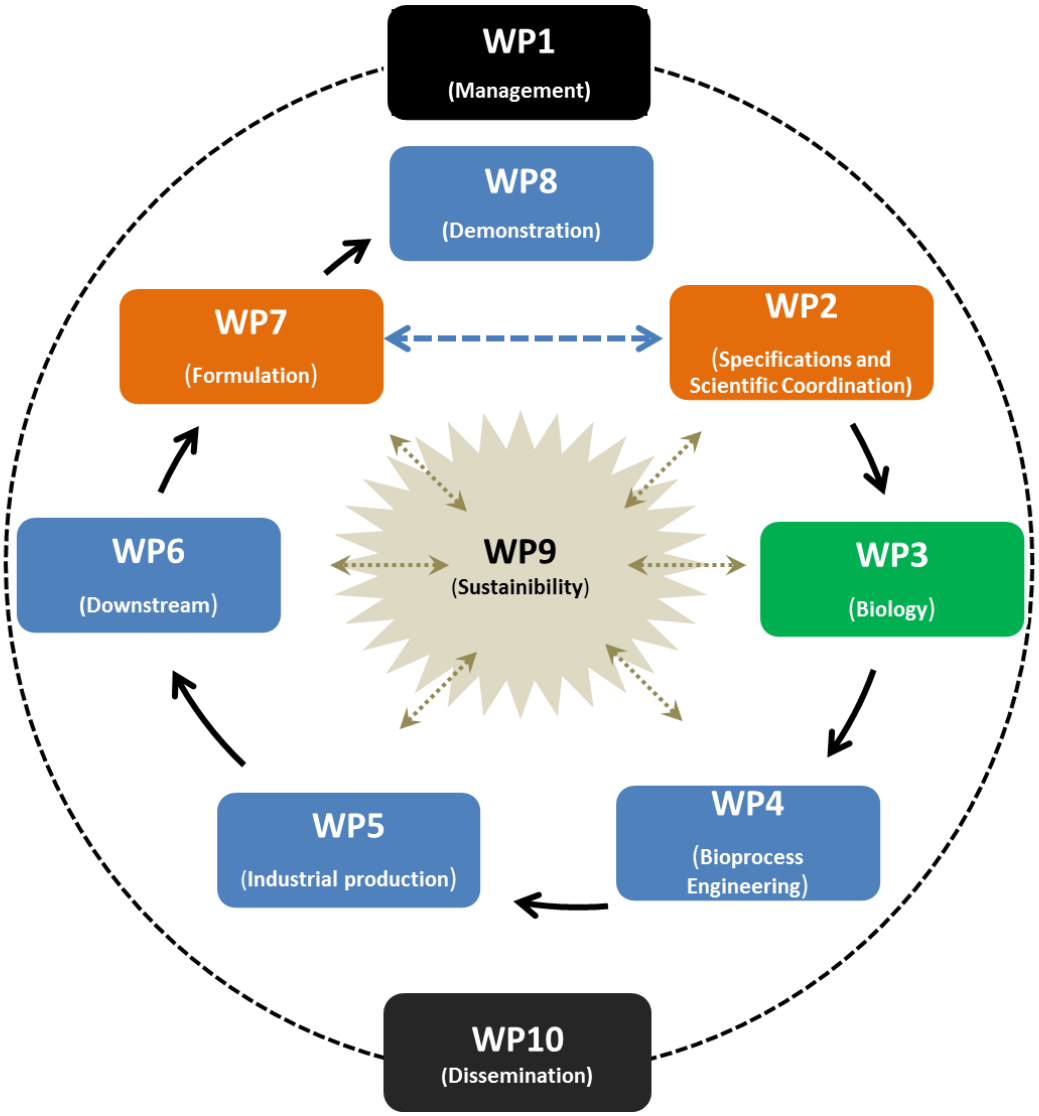

Fig. 1. Value-Chain Concept as PERT diagram (End-User / Biology / Technology)

3. Main Scientific and Technical Results

Following the valuable chain concept and targeting innovative product magnesium /calcium salts with 95% EPA or 95% DHA, or mixture of the EPA and DHA PUFAChain project achieved following results. Novel for the pilot production scale EPA and DHA producing algae strains were tested in combination with novel PBR design and whole year operation (Algae-Crop-Rotation principle). This was combined with pre-pilot scale tests of the extraction technologies that are well-established for other objects neutral lipids extraction (scCO2 and propane extraction). Additionally, novel (enzymatic cleavage) in combination with established (short path distillation) purification steps were tested on the model oils and crude algae oil extracts.

3.1 WP 2 Specifications and Scientific coordination

WP2 includes the project specifications and scientific coordination of the project. The objectives were to develop a target product profile (end-user specification) for Omega 3 fatty acids suited for the synthesis of esterified downstream products to be applied in pharmaceutical, nutritional and cosmetic consumer products. Also, develop analytical methods to characterize target compounds and by-products in complex matrices. Outline the complete supply chain from algae to the end product and define operating procedures (SOPs) to meet the target specification. Making sure that the project is on time and that all deadlines for milestones and deliverables are met properly and organisation of the scientific part of the project meetings.

3.1.1 Work Undertaken

Partners formulated the following requirements, in order to guide strain selection and screening during the WP3 Biology.

Requirements concerning the PUFA type (IOI, A4F):

- high EPA and/or DHA content/yields (>1% d.w. EPA, and presence of the DHA);
- low or better no content of arachidonic acid (ARA) to facilitate refinery and separation of EPA and DHA by the IOI Oleo approach of enzymatic splitting (>30% of the EPA content in the cell);
- no excessive amounts of non-acidic or other by-products (e.g. fucoxanthin, xanthophylls, anthocyans, extrapolymeric substances) that might interfere with refinery process.

Requirements of strains regarding mass culture conditions (A4F):

- suitable for mass culture in horizontal tubular photobioreactors (TPBR) (unicellular or short filaments, cell wall present, etc.);
- non-sensitive to pumping which is used for circulation in PBRs;
- no negative effects when aerated with air plus 2% v.v. CO_2;
- preferably suitable for specific A4F culture medium (freshwater medium);
- preferably no vitamin requirements (e.g. vitamin B_{12});
- high productivities of EPA/DHA at ambient temperatures throughout the year in semi-continuous or batch culture mode.

Harvest/processing requirements (MIN and NTX):

- suitable for harvesting by cross flow membrane microfiltration;
- the strains should not produce any extrapolymeric substances (EPS) as such EPS would most probably interfere with filtration units and block flow;
- spray-drying and pelleting of algal biomass for easier cell disruption and handling of dry biomass; alternatively a belt solar dryer might be used;
- cell disruption by pulsed electric field (PEF) or osmotic shock;
- extraction of wet biomass by propane and/or of dry biomass by supercritical CO_2.

Based on the activities in other Work Packages the SOP were developed for the (1) process conditions to guarantee high quality and yield; (2) harvesting conditions with respect to water content, oxidative degradation (air contact) to exclude possible damage; (3) transportation and storage conditions; (4) specification parameters for end product (Omega-3-fatty acids) and intermediates and develop suited analytical methods to characterise target compounds and by-products, (5) parameters for the characterisation of the raw material - algae cell biomass which will be extracted by supercritical or liquid.

3.1.3. Impact on the other work packages.

Algae strain screening and selection in WP3, and subsequently work in the bioprocess engineering WP4 and downstream Work Packages WP6, WP7, WP8.

3.2 WP3 Biology

The work in the work package Biology was focused on the optimization of pre-selected strains from the UGOE culture collection; optimization of strains for the “algal crop rotation" (ACR) principle for production; unambiguous identification of algal strains to ensure their high quality; maintenance of the project’s algal strain database and provision of a genetically stable stock through cryopreservation.

Optimization of pre-selected strains from the UGOE culture collection

More than 160 strains were preselected based on their quantitative lipid profile for further experiments. The strains that showed good growth under standard conditions were selected for the quantitative lipid profile analysis. Algae were screened for the quantity of the EPA and DHA in the lipid profile, and diversity of the lipid profile (how many and which lipid species are present).

A total of 96 algal strains from both bioresources at UGOE (SAG; 52 strains) and Fraunhofer (CCCryo; 44 strains) was selected because of growth properties appeared appropriate for both biotechnological mass cultivation and PUFA content. Based on the initial list of potential algal strains a selection of 22 strains after PUFA/metabolite testing was made which will be considered for further analyses. The selection includes the nine “best” EPA only producing strains, i.e. nine Stramenopile algae from the UGOE growth experiments. Except for a single strain, these strains represent species which have not been exploited for biotechnological purposes so far (“novel strains”). The selection also includes the three species which were found to exhibit EPA and DHA in almost 1:1 ratios, but at rather different concentrations. Five DHA only-producing strains were selected based on their DHA concentrations, although in none of the strains the DHA content is satisfactory. Finally, also five EPA producing strains were considered despite they contain considerable amounts of ARA because they represent cold and low light adapted species which may be important to realize the “algal crop rotation" (ACR) principle.

Optimization of the biomass and lipid content. The target EPA and DHA in the algae cells are distributed between different classes of the polar and neutral lipids. The total lipid content can be manipulated with nitrogen availability, especially neutral lipids, but the increase of the neutral lipids does not lead to the increase of the EPA and DHA content in the algae biomass. Therefore, we focused on the biomass production improvement, and consequent increase of the EPA and DHA yields. More than The productivity of the selected algae strains can be improved with manipulation of the phosphate content in the medium and addition of the 2% v.v. CO_2 to the aeration. The addition of the CO_2 to the aeration and change in the temperature conditions can drastically change the algae response to the cultivation medium. For the EPA producing strains from the Eustigmatophyceae the Kuhl medium, with reduced phosphate content and 2% v.v. CO_2 (pH 6 – 7) was the

most appropriate medium for the biomass accumulation. The *Prorocentrum* showed best growth on the mixture of the ASM30 and seawater, at temperature 21 - 25°C and addition of the 2% v.v. CO_2 (pH 7.5 – 8).

Small scale screening (up to 50 ml) does not have enough of the predictive power for the upscaling process. In the future, we would suggest adding analysis of the distribution of EPA and DHA between polar and neutral lipids, to the qualitative and quantitative lipid profile analysis.

Unambiguous identification of algal strains to ensure their high quality

Taxonomic revision of Vischeria – Eustigmatos clade of the EPA producing strains from Eustigmatophyceae. Clarification of the taxonomy of these organisms at the species level is important to achieve reproducible results and yields of valuable compounds in their exploitation. The distinction of the so far exclusively morphologically defined species of the genera Eustigmatos and Vischeria was tested utilizing all available authentic reference strains to enable taxonomic assessments. DNA sequence-based distinctions from the ITS2 rDNA region (23 strains), almost full 18S rRNA (6 strains) as well as plastid-encoded rbcL (9 strains) gene sequences were evaluated and contrasted with a reassessment of morphological features of the studied strains. The secondary structure-based phylogenetic analysis of ITS2 clearly separated independent lineages (species) forming a star-like tree topology, but no groups of strains were resolved. Therefore, morphological features as previously used to separate two genera were revealed inadequate. Consequently, the clade of closely related species which was well supported by ITS2 secondary structure features as well as in the 18S phylogenies, is regarded as representing a single genus, Vischeria. Taxonomic changes among the species with the definition of epitypes on the basis of cryopreserved strains were recommended. For three species intragenomic ITS2 variation was revealed, but the paralogues were closely related (except for one species). The disagreement between nuclear and plastid markers as well as the extent of intragenomic ITS2 variation needs to be further investigated because they may bear significant information for understanding species evolutionary history. Also, species diversity within Vischeria needs to be further explored. It appeared still unsaturated because (except for a single case) no two isolates were found that represented the same species.

Optimization of strains for the “algal crop rotation" (ACR) principle for production

One integral part of the PUFAChain project was the idea to test and implement the so-called "Algae Crop Rotation (ACR)" principle as a novel mass culture technique to increase the overall economic efficiency of an algal production process. With this we aimed to solve the problem that many outdoor photobioreactors become uneconomic for a specific period of the year, when growth rates and metabolite production of the specific algae strain cultured decrease due to environmental parameters changing to unfavourable conditions. Switching from one strain to another, each with different requirements regarding temperature and/or light, and by this remaining productive during the complete time of the year, was the hypothesis to be tested.

Scientific Results

For proving the ACR principle (a detailed description can be found in Deliverable D3.2), first, the CCCryo Culture Collection of Cryophilic Algae was screened for the best performing strains with regard to EPA and DHA production under cold temperatures. In Deliverable D3.3 (Project’s algal strain database) all 68 CCCryo strains are listed that were screened. With regard to their temperature requirement they are marked as either being mesophilic, cryotrophic or psychrophilic. It showed that only two strains of *Cylindrocystis brebissonii* and *Lepocinclis globulus* reliably produced DHA. Due to extremely low growth rates these were not further investigated in this project. Instead, various strains of the psychrophilic *Raphidonema nivale* proved to produce acceptable amounts of EPA under cold conditions. Four of them (CCCryo 274-06, 359-10, 375-11 and 381-11) showed best performance regarding highest EPA content and productivity. Finally, strain CCCryo 381-11 was chosen as the most promising strain for an EPA production under cold conditions or as a "rotating crop" within the ACR principle. This strain also contained least amounts of arachidonic acid, which was not desired in high contents in the final product.

We then compared this psychrophilic strain of *Raphidonema nivale* with the mesophilic strain of *Chloridella simplex* (SAG 51.91 = CCCryo 175-04). Though this *Chloridella* strain also was isolated from a snow sample our studies showed that both strains prefer very different both temperature and light conditions. *C. simplex* shows

highest EPA yields at temperatures of 20-30 °C, *R. nivale* instead at 4-20 °C. *C. simplex* also prefers high PAR photon flux densities of 30-1,000 µmol m^{-2} s^{-1}, whilst the *R. nivale* prefers low light conditions of 5-100 µmol m^{-2} s^{-1}. We studied the two strains at different temperature and light conditions and modelled their EPA productivity along a temperature profile at 4, 8, 12 and 20 °C representing a gradient along different North, Middle and South European locations and their seasons respectively (see 4th periodic report). We could show that at 4 °C biomass productivity is 4-fold higher in *Raphidonema* than in *Chloridella* (winter conditions). With rising temperatures this value decreases in *Raphidonema* and increases in *Chloridella*. At around 12 °C biomass productivities in both strains equal out (spring/fall temperatures). Above this temperature *Chloridella* is a better biomass producer than *Raphidonema*, however, at 20 °C (summer conditions) it does not even reach *Raphidonema's* productivity at 4 °C. Looking at the EPA productivity and taking the ability of *Raphidonema* to be highly productive under low light conditions into account, again, *Raphidonema* produces considerably more EPA at low temperatures than *Chloridella*, up to approx. 3-fold at 4 °C with additional CO_2. However, at temperatures above 12 °C and especially under summer conditions *Chloridella* is a much better EPA producer than *Raphidonema*.

Summarised, each strain has its strength under either low (winter) or high (summer) conditions, and this is worth to be considered when planning production processes. An algae production plant in Northern Europe will not be able to produce any EPA during the colder months when solely growing *Chloridella* or presumably any other mesophilic strain. For the darker and colder seasons he would be well advised to grow a cryotrophic or psychrophilic alga such as *Raphidonema*, which would produce at least some EPA, even though not as much as he can produce with *Chloridella* in summer. Without a winter-compatible strain he would have to stop algal production completely during the colder and darker months. A facilitated diagram taken from Deliverable D3.2 is shown in Fig. 2.

The crux of the matter and the weakness of our proposed ACR principle currently still lies in other facts. The principle itself works and we could prove it. However, when taking further production costs like those for power (mixing, harvest and also occasional heating during winter to prevent the plant from freezing) and CO_2 into account, these are not counterbalanced by the extra gain from EPA production during that time (compare Deliverable D9.4). In several aspects the production of a psychrophilic alga in Northern Europe is advantageous over using a mesophilic one, but overall under the current conditions it does not pay in the end. As summarised in D3.4 the overall costs for 1 kg EPA produced in Southern Europe lie around 1,000 EUR when using *Chloridella*. With an optimistic view these costs increase to nearly 4,000 EUR when producing EPA in Northern Europe using *Chloridella* and *Raphidonema* according to the ACR principle due to extra energy costs. On the other hand it has to be said that these values result from models. Especially costs for energy and manpower can vary considerably between locations/countries. An optimisation of the algal culture process itself could further increase its productivity, as we could already show with additional CO_2, which led to more than doubling of EPA yields. Energy costs in some North European countries or specific locations can be lower than calculated in our models e.g. due to the availability of cheap geothermal energy (Iceland) or hydro power (Norway) or free CO_2. This also would reduce costs from a sustainability point of view.

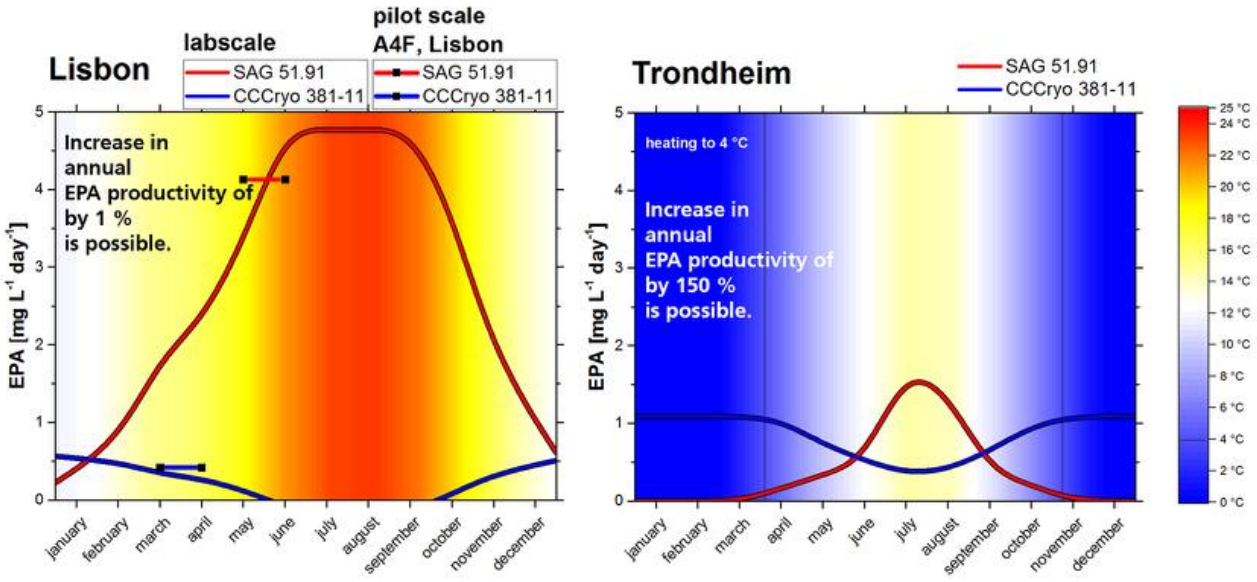

Fig. 2. The ACR principle at two different locations. *Chloridella simplex* strain SAG 51.91 is a typical summer strain and should be cultured at locations in South Europe (e.g. Lisbon), however at North European locations it will only produce EPA during a couple of summer months. To remain productive during the colder seasons of the year psychrophilic algae such as the *Raphidonema nivale* strain CCCryo 381-11 should be employed.

As outlined in the SWOT analysis of the socio-economic assessment in Deliverable D9.4, PUFAChain in general and the ACR principle in the same way still have systemic weaknesses: the energy consumption for algae production still is very high as a result of a still immature production process, and it weighs too heavy on the overall costs compared to the gain from the final product and often also in comparsion to traditional sources. As depicted in Fig. 3, energy input has to be reduced while the productivity of the whole process has to be increased to become competitive.

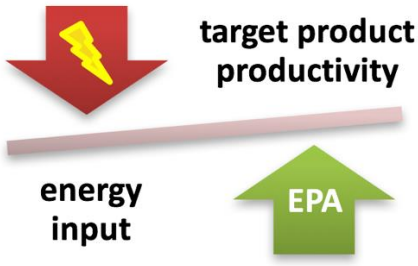

Fig. 3 Algal production processes in general still lack efficacy. Production processes are functional, but still too expensive to compete with products from traditional sources.

Recommendations to enable a profitable ACR principle:

- reduce production costs (OPEX);
- grow well characterised algae strains during their preferred seasons of the year, i.e. mesophilic strains in summer, cryophilic ones in winter;
- develop less cost intensive methods for harvest and downstream processes;
- reduce running costs (CAPEX), especially energy costs, by using cheaper/renewable resources, i.e. plan algae plants at respective countries/locations;
- and/or increase EPA productivity in algae strains;
- optimise strains by selective culture (more productive, less prone to contaminants. etc.);
- improve production process (culture medium, CO_2 addition, mixing, illumination/natural irradiation).

Impact on other Work Packages

Results from the above task were used as input for WP5 and WP9. In WP5 production and productivity values from experiments run at Fraunhofer and A4F were used to deliver data to plan an industrial scale production facility including downstream processes. In WP9 these data were used to allow an as precise as possible assessment of technological indicators, environmental impact and socio-economic aspects resulting in a final integrated assessment.

3.3 WP4 Bioprocess Engineering

3.3.1 Work Undertaken

The main objectives for the production at pilot scale were: (i) test the selected strains with potential for high added value products in a pilot scale plant (1 to 10 m^3), to be optimized in a biorefinery logic; and (ii) provide specific details under best operational conditions and technology optimization for scale-up purposes. In order to provide operating data for the engineering and design, and specific information on productivity and operational parameters, for an industrial plant, the whole production process configuration within the pilot unit was established.

The work developed in WP4 is divided in three tasks: (1) biomass optimization; (2) scale-up from 10L to 10,000L; (3) biomass pre-processing for further downstream procedures.

Biomass optimization. The purpose of this task was based on a two-step approach. In a first stage the selected strains from WP3 and an additional strain from A4F, already known from the industry, were cultivated at laboratory scale at A4F. The characterization of the microalgae at a 5 L scale highlighted and benchmarked biomass productivities and PUFA content of the products of interest, omega-3 EPA and DHA, and also omega-6 arachidonic acid (ARA) a fatty acid under scrutiny in the project. The data collected regarding the performance of the several strains was used to select strains for pilot scale tests.

Following the information gathered from the above-mentioned laboratory assays, strains production at pilot scale was aimed, according to their potential of success. The six selected strains were produced with success at pilot scale at A4F Experimental Unit. These strains included a mesophilic algae from existing industrial exploitations that was used for benchmarking, establishing a solid reference and standard of comparison for the goals of the PUFAChain project, as well as 4 other mesophilic strains and 1 cryophilic strain from the culture collections of the project partners.

Scale-up from 10L to 10,000L. This task included the activities necessary for the engineering design and expansion of the Pilot Unit in Lisbon, its implementation project and cultivation activities, in order to achieve the scale-up of production from 10 to 10,000 L. The Prototype Unit expansion project was based on a process concept, starting with a high-quality inoculum to be produced in photobioreactors (green walls), followed by a growth phase (tubular reactors or cascade raceway) and finally the operation of the photobioreactors in a biomass production in a regular basis. One of the main principles behind this project is the versatility which, by installing different technologies, allows testing different production scenarios in order to determine the optimal cost-effective technology to the desired products and also produce biomass for further processing by PUFAChain partners.

Biomass pre-processing. Biomass was harvested by a membrane system developed and provided by MAHLE InnoWa and the facility prepared to recirculate the culture medium after treatment for restoring its quality for algae growth.

The data gathered at pilot scale in A4F was then compared with the calculations and estimation performed by MAHLE InnoWa.

3.3.1 Scientific and Technical Results

Strain selection. The six strains that were produced at pilot scale in A4F Experimental Unit, were:

- three strains well known from the microalgae industry, including two from project partners culture collection (as reference strains): CCAP 1085/18 *Thalassiosira weissflogii*; SAG 1090-6 *Phaeodactylum tricornutum*; SAG 2.99 *Nannochloropsis gaditana*

- three novel strains: SAG 40.80 *Prorocentrum cassubicum*; SAG 51.91 *Chloridella simplex*; CCCryo 381-11 *Raphidonema nivale*.

The six selected strains were produced with success at pilot scale at A4F. Information gathered from over 30 pilot scale trials and with a combined total of more than 1200 cultivation days provided the necessary data for comparison of biomass and PUFA productivity between strains. Assays performed included testing and optimization of various cultivation systems (and regimes), several strategies for lipid production (e.g. high light and nitrogen starvation) and different salinities, among others. Based on the data obtained, the recommended culture conditions for production at pilot scale were determined for each strain selected by the consortium with highest potential in the context of the PUFAChain project.

Results obtained for normalized PUFA productivities, taking into account biomass productivity and the lipid profile content of each strain, provided the means to establish the strains better suited for EPA and/or DHA production, coupled with a low ARA content. Best determined EPA producers were *C. simplex* and *N. gaditana* and best DHA producers were *P. cassubicum* and *T. weissflogii*. *R. nivale*, being a cryophilic strain, is an alternative EPA producer during winter conditions, allowing for the application of the ACR (Algae crop rotation) principle.

Technology parameters for biomass production at Pilot Scale

Knowledge obtained during pilot scale trials allowed the selection of the most suitable technologies to implement in the Demo Plant:

GW for the scale-up of the laboratory inoculum, allowing the production of inoculum in a *batch* cultivation mode to be used to inoculate the production systems;

TPBR for microalgae production under controlled parameters, allowing for the successful production of biomass in a semi-continuous regime during long periods of time (over 100 days in production).

Furthermore, following the selection of the TPBR as the most suitable technology for production in the scope of the PUFAChain project, **A4F successfully adapted the design, built and tested an optimized system for the PUFAChain: the Unilayer Horizontal Tubular Photobioreactor (UHT-PBR)**. **This technology significantly lowers capital expenditures (CAPEX), as well as operational expenditures (OPEX)**, when compared with previously used Multilayer Horizontal Tubular Photobioreactors (MHT-PBR).

Technology parameters for the MAHLE membrane system

Technology parameters for the MAHLE InnoWa membrane system were applied at A4F Experimental Unit in Lisbon. The estimated and calculated results were confirmed under real conditions with the pilot test set-up. By using the MAHLE crossflow-system the project partners were able to omit a centrifugation as an additional harvesting and dewatering step that can decrease investment and energy costs. Another important point is the possibility of a significant amount of water reuse <any numerical data> by using the filtrate of the harvesting step as breeding media for the algae reactors. Furthermore, the crossflow system is easily scalable to the desired size of installation, namely for the Demo Plant and Industrial Facilities.

During the project MAHLE InnoWa developed a new membrane in their laboratory and built up modules for the experimental set-up. They have determined a clean water flux for the membrane first, which coincides with the demands of algae filtration and suits all requirements such as high solid content and salt- water. These modules, respectively the new fibers, worked excellent in accordance to the special conditions in the fields of algae filtration, such as high particulate solid and salt water. Beyond that, it is possible reduce the membrane fouling, particularly by using the appropriate cleaning agents <any numerical data>.

The third point is the interaction between the membrane and the filtration system in the case of the cleaning of the system. MAHLE InnoWa has invented an additional cleaning procedure having a de facto reversion of the flow inside the modules. When cleaning the system, in this particular case with warm water, a high endurance of the crossflow system can be achieved and save cleaning agents. Under these process conditions, cleaning agents can be saved and production downtime avoided.

Operation procedures for Pilot Unit operation

With the conclusion of the Experimental Unit expansion project it was possible to define the procedures for all systems implemented, which allow for the successful optimized cultivation of all the strains selected in the scope of the PUFAChain project. Protocols were developed for every procedure from material, solutions and cultures preparation and monitoring to their disinfection and cleaning. The operational procedures were detailed for the following subjects: 1. Medium preparation and storage; 2. Culture carbonation; 3. Inoculum preparation; 4. Production optimization and scale-up; 5. Culture thermoregulation; 6. Laboratory operations; 7. Harvesting and pre-processing; 8. Water recycling and wastewater treatment; 9. Data acquisition. These procedures are required to implement and operate the microalgae production facility of the DEMO Plant.

3.3.3 Impact on other Work Packages

Work developed on this WP benefits from the inputs from WP3. The work developed in this WP provided key inputs for WP5, namely data regarding: biomass productivities, biological constrains of the algae and technology, mass and energy balances, production and pre-processing process optimization, development of protocols required to implement and operate the microalgae production facility of the industrial Unit, and base investment and operation costs. Also, this WP provided the necessary information for the Life Cycle Analysis in WP9.

Even more, biomass produced at pilot scale in the scope of this WP was sent to project partners responsible of WP6 and WP7 for extraction and/or purification tests.

3.4 WP5 Industrial Production

3.4.1 Work Undertaken

The two main objectives was (1) to provide the pre-engineering project design for an industrial facility for microalgae biorefinery, by gathering available knowledge from partners and coordinate the design efforts along the entire project, so that the most adequate technical solutions found are applied and integrated in the whole process; and (2) to develop a business plan that define the technological and economical constrains of the biorefinery facility and prepare documentation, so that investors can be attracted to the project.

The work developed in WP5 is divided in four tasks: Short-list of the best locations for an industrial facility for microalgae biorefining; Pre-engineering project for the construction of an industrial facility for microalgae biorefining; Business plan for the demo plant and a future industrial facility; Supercritical/liquid solvent extraction.

Short-list of the best locations for an industrial facility for microalgae biorefining

The methodology applied to define the crucial criteria was to interview several experts in the field about what are the best criteria to choose a location for microalgae biorefinery for PUFA. Three criteria were selected: (1) the pre-existence of a microalgae farm in the surrounding area so that the industrial facility can be supplied with the required raw material; (2) the existence of knowhow that can be used to create, operate and upgrade the biorefinery; and (3) the proximity to possible consumers of the end product. To these three criteria, a fourth criterion is proposed by A4F team that complement the ones previously mentioned, which is the PESTLE (Political, Economic, Social, Technological, Legal and Environmental) analysis.

Pre-engineering project for the construction of an Industrial Facility for microalgae biorefining

In order to execute the pre-engineering project of the Industrial Facility, dimensioning of the respective Demo Plant was performed and analysed for each scenario. The size of the Demo Plant and the Industrial Facility to be designed was defined by the PUFAChain consortium as 10 and 100 ha of production area, respectively.

The pre-engineering project of the Demo Plant and Industrial Facility designed for the project includes (1) the definition of the global characteristics of the plants, including plant sizing; (2) the process flow-sheeting; (3) the detailed mass balance and energy balance of each studied scenario; (4) the detailed layout of the plants, including all the process units and ancillary facilities; (5) the specification and design of the ancillary services; (6) the global equipment selection specifications and design and (7) the preliminary cost estimations.

Business Plan for the Demo Plant and a future Industrial facility

A comprehensive business plan for the plants designed in the PUFAChain project was performed in this task, which includes (1) a description of the several approaches and scenarios being studied; (2) the characteristics of the plants being studied; (3) a list and justification of the assumptions in which the business case is based; (4) characterization of the PUFAChain products for the business case; (5) a description of estimated capital and operational costs for the production plants being considered and (6) an estimate of the capital revenues for each approach and production scenario. The project was prepared to be presented to possible investors including the investment highlights, the economic analysis and the key milestones for the microalgae biorefinery plant and a roadmap was designed for future exploitation of the developed business plan.

3.4.2 Scientific and Technical Results

Best locations for the PUFAChain Biorefinery

The most two attractive locations for the PUFAChain Biorefinery are in Portugal and in Germany since the PUFAChain consortium has the information and the expertise to consolidate the Business Plan and the Life Cycle Analysis in these two countries. Moreover, to analyse the algae crop rotation (ACR) principle, which is one of the novelties of the project, two different latitudinal belt in terms of annual solar irradiation and annual temperature should be considered, one in Southern Europe and the other in Central Europe. Included in these two latitudinal belts are the regions of Lisboa in Portugal and Munich in Germany. The main advantages of these locations are (1) proximity to technology and logistics for microalgae production and biorefining; (2) easy access to the most relevant raw materials and utilities; (3) easy access to all transportation systems; (4) availability of labour and a local talent pool; (5) well-known political strategies; (6) close to the potential market

Scenarios for the Demo Plant and Industrial Facility for the PUFAChain Biorefinery

The units design is based on (1) the same process concept applied in A4F Experimental Unit in Lisbon, the outcome of which followed the selection of the most suitable technologies to implement in the DEMO plant: Green Walls (GW) flat panel photobioreactors for inoculum scale-up and tubular photobioreactors (TPBR) for biomass production and (2) expertise and data provided by each of the project partners involved in WP5, WP6 and WP7 (A4F, MAHLE, Natex and IOI oleo) for the dimensioning of the culture processing, extraction and oil processing systems.

For the Demo Plant designs, a conservative approach is considered, assuming the least expected performance in cultivation productivity as well as losses during biomass processing. In the Industrial Facility scenarios, an optimistic performance is favoured, in which culture productivity is higher and processing losses are further minimized. With the information gathered during the project, the Demo plant and Industrial Facility scenarios selected by the PUFAChain consortium and analysed in this WP were:

- SEDL – **S**cenario of **E**PA+DHA production in **L**isbon (*Prorocentrum* cultivation)
- SEDM – **S**cenario of **E**PA+**D**HA production in **M**unich (*Prorocentrum* cultivation)
- SEL – **S**cenario of around the year **E**PA production in **L**isbon (combined cultivation of the *Chloridella* and *Raphidonema*)

• SEM – **S**cenario of around the year **E**PA production in **M**unich (combined cultivation of the *Chloridella* and *Raphidonema*)

Considering the market prices, the annual production for each product expected in each of the final scenarios considered and the CAPEX and OPEX values for these scenarios, the economic analysis was developed with the support of a sub-contracted external company. In the final analysis two strategies were applied to the business plan of the Industrial Facilities for each scenario. (A) The first one was focused on finding new product values or new product formulations that could increase the total revenue of the biorefinery microalgae unit. (B) With the second strategy, the microalgae biomass produced was considered to be transported to a well-established biorefinery plant which ensures the best use of the extraction and oil processing facilities for the biomass processing. In this strategy, there is a decrease on the investment costs.

The key conclusions obtained from the economic analysis of the 100 ha PUFAChain biorefineries are:

- In all of the business to business strategy scenarios that were analysed, for both Lisbon and Munich plants, the NPV value obtained is negative and payback periods are over 20 years. These scenarios are therefore not economically attractive for potential investors for the project. With the considered CAPEX and OPEX data, economically interesting scenarios can only be achieved with higher profits from product sales, which is only feasible in a business to consumer approach;

- Production scenarios in Lisbon are more profitable than the ones presented in Munich. This is due to the comparatively higher production capacities on this location, due to the generally more adequate climatic conditions for microalgae production;

- The scenarios for the production of EPA and DHA are more economically attractive when compared with the respective EPA only production and this applies for both locations. The difference between these two production scenarios is much higher for the Lisbon location. This is mainly due to the application of the ACR principle, from with the Munich location benefits more with the considered project strains;

- The best scenario is SEDL in a business to consumer strategy, with a payback period of 6 years, as well as the highest values for EBITDA, IRR and NPV indicators, which is a very appealing profile for potential investors.

According to the PUFAChain project main goal, PUFA production, the main conclusion is that for the technological options of the project, a production unit is possible and economically feasible, within a biorefinery logic. This is achieved for both the EPA and DHA and EPA only productions strategies and for both locations considered.

Project presentation and roadmap for future exploitation

For the dissemination of the developed business plan of the PUFAChain biorefinery and in order to find an investor for the project, documents were prepared detailing the PUFAChain biorefinery project, including the investment highlights, the economic analysis and the key milestones for the microalgae biorefinery plant was prepared.

As a cutting-edge biorefinery project, PUFAChain presents a unique investment opportunity for investors interested in nutrient ingredients and pet food specialty ingredients derived from a sustainable feedstock. This investment opportunity's top five value propositions include (1) Leading microalgae biorefinery solution provider; (2) Value-added product portfolio and a strong market outlook; (3) Access to one of the leading networks of algae experts in Europe; (4) Flexibility in project configuration; (5) Access to low-interest loan guaranteed by the European Investment Bank.

Conclusion of the project and transfer to investor for future exploitation

Despite the effort spent in optimization of the Business Case and the analysis of various scenarios, with the evaluation of different strains, locations and production size, and despite the preparation of the project to be presented to possible investors, the PUFAChain project can only be ready for Investment phase in a timeline after the PUFAChain FP7 project has finished.

Therefore, a roadmap was designed for future exploitation of the Business Case that was developed. The roadmap designed consisted in the following phases (1) Project timeline; (2) EEIG registration; (3) Finding an investor; (4) Construction and commissioning phase and (5) Exploitation.

3.5 WP6 Downstream

3.5.1 Work Undertaken

Cell disruption. Several methodologies were investigated for each strain within the scope of the PUFAChain project, in order to develop an effective cell disruption method for algal biomass, finding the most promising one for the pilot scale pre-processing of the produced biomass. The tested methods at A4F included: pulse electrical field (PEF), glycerol treatment, acid treatment, osmotic shock, freeze/thaw cycles, sonication and mechanical stress.

Crude oil extraction by organic solvents. With the objective of developing novel extraction processes for isolation of the lipid fraction from the algal biomass, A4F tested oil extraction in SAG 2.99 *N. gaditana* and SAG 51.91 *C. simplex* biomass using a transesterification methodology involving the use of organic solvents ethanol and hexane. To complement work developed in this subject, A4F continuously developed a method to evaluate cell rupture and cell content extraction availability. It acted as a first proxy for the performance of each cell rupture method and to establish relative ease of extraction between strains and methods tested.

3.5.2 Scientific and Technical Results

Cell disruption. In the final stages of the project, four strains were agreed upon by the consortium as having the most potential in the scope of the PUFAChain project, namely SAG 40.80 *P. cassubicum*, CCAP 1085/18 *T. weissflogii,* SAG 51.91 *C. simplex* and CCryo 381-11 *R. nivale*. For this reason, during the last months of the project, the focus for cell disruption tests at A4F was on these 4 strains. Cell disruption methods for SAG 40.80 *P. cassubicum* and CCAP 1085/18 *T. weissflogii* were achieved with >95% efficiency under mild conditions. Considering the SAG 51.91 *C. simplex* and CCryo 381-11 *R. nivale* cultivation scenarios, none of the tested methods was satisfactory in providing an efficient solution for both strains. For this reason, a standard method such as bead milling was considered to be the most appropriate method for cell disruption and to be considered in the scenarios analysed for WP5 and WP9.

Crude oil Extraction. The extraction of SAG 2.99 *N. gaditana* and SAG 51.91 *C. simplex* biomass was successful using a transesterification methodology, with 60 to 70% of initial fatty acids recovered in the form of fatty acid ethyl esters for both strains. Fatty acid profile obtained was very similar to the original samples. This method has the advantages of being able to be applied without previous cell rupture being required, as well as the simultaneous extraction of both polar and non-polar fatty acids in the biomass. However, it also leads to significant compound degradation of the biomass, decreasing the overall biorefinery potential of this method. For this reason, other extraction methods were favored to be used in WP5 and WP9 scenarios.

Liquid solvent extraction. The work package focused on developing a viable, robust and efficient processing for the algal biomass to produce highly purified algal cell components, especially polyunsaturated fatty acids. In order to successfully fractionate the targeted compounds from the algal biomass, a cell disruption is necessary to provide access of the extracting solvent to the compounds. A variety of different cell disruption methods was tested on selected microalgae strains. It can be concluded, that for each algae strain, a specific cell disruption method has to be developed and tested. Among the evaluated cell disruption methods, the osmotic shock during the diafiltration, disruption effect of the glycerol on the cell membranes and sonication were the most applied. Some uncertainties in the measurements of the effectiveness of the tested cell disruption methods were caused due to the fact, that the target molecules are unstable and degrade already during transportation of the samples.

Extraction of crude oil by organic solvents was used to measure the efficiency of used cell disruption methods and also to quantify the amount of oil in the produced biomasses in the project. The methods were not used to directly produce crude oils for further downstream treatment.

Extraction of crude oil from dried algae biomass by supercritical CO_2 (Fig. 4) was used to produce crude oils from different dried microalgae biomasses. Extraction with supercritical carbon dioxide is a well established, industrially applied technology for production of many different products, also special oils from sources like roasted sesame oil, cosmetic oils, waxes etc. The main advantage of this technology is the fact, that only compressed carbon dioxide is used as a solvent, which means, all products and spent materials are free of organic solvents. In total, 34.9 kg of dried powder (~26.9 kg of dried algae biomass) from different strains was extracted by supercritical CO_2 during the project, yielding in 1.382 kg of crude extract. Phaeodactylum (11.1kg) 304.6g 2,74% extract yield. Thalassiosira (12.3 kg) 587.0 g 4,78% extract yield, Prorocentrum 2.5 kg 78.0 g 3.02% extract yield, Raphidonema 2.5 kg 192.0 g 7,45% extract yield.

Fig. 4. 5L/1000bar supercritical CO_2 extraction unit in Natex laboratory

The extracts were consisting of crude oil, water, pigments and other minor impurities. The fractions of crude oil from the extracts were analysed on fatty acids profile and acid values. Overall, the fatty acid profiles were similar to those from lipids in biomass (Table 1).

Table 1. Examples of fatty acid profiles of the CO_2 extracted microalgae oils

FA (in mg/ g oil phase)	Phaeodactylum (NX3010)	Prorocentrum (NX3258)	Thalassiosira (NX3282)	Raphidonema (NX3284)
Myristic acid	81.4 ± 6.4	19.8 ± 0.9	47.1 ± 2.7	8.9 ± 0.3
Σ C16	338.1 ± 8.2	115.7 ± 14.4	406.1 ± 23.8	224.9 ± 8.7

Σ C18	48.2 ± 1.6	196.5 ± 9.3	38.1 ± 2.7	381.3 ± 21.1
Stearidonic acid	n.d.	121.5 ± 6.6	8.0 ± 0.7	29.9 ± 0.9
Σ C20	-	165.7 ± 8.4	216.4 ± 13.6	212.9 ± 3.1
Arachidonic acid (ARA)	57.7 ± 2.3	n.d.	n.d.	33.4 ± 0.3
Eicosapentaenoic acid	182.7 ± 10.1	165.0 ± 8.4	216.4 ± 13.6	165.3 ± 3.0
Σ C22	N/A	124.3 ± 6.0	85.8 ± 0.5	1.1 ± 0.1
Docosahexaenoic acid	N/A	121.7 ± 6.0	85.8 ± 0.5	n.d.
Σ Others	N/A	7.0 ± 0.2	9.3 ± 0.5	7.5 ± 0.0
Total	-	629.1 ± 12.0	802.8 ± 43.8	836.6 ± 33.2

The extracts contain relative high amounts of the target polyunsaturated fatty acids, e.g. 216 mg of EPA/g oil of the *Thalassiosira* extract. The acid values of the oils were in all cases very high, indicating high degree of hydrolysis of the triglycerides. Last results indicate fast enzymatic hydrolysis of the lipids during the harvesting procedures.

The knowledge of extracting the lipid fractions from dried microalgae biomass gathered in the course of the Pufachain project can be summarized in the following points:

- Neutral lipids (TAG's) are well soluble in $scCO_2$ and can be extracted. Triglycerides containing polyunsaturated fatty acids can be extracted.
- Pigments like carotenoids can also be extracted.
- Polar lipids like phospholipids cannot be extracted. In some microalgae strains, the polar lipids contain part of the polyunsaturated fatty acids, so they cannot be extracted by $scCO_2$.
- Stability of the microalgae biomass – after harvesting of the biomass, the cell lipases become active and hydrolyse the triglycerides, which leads to undesired product. Enzyme deactivation step is necessary.
- After harvesting, most of the biomass is spray dried which leads to a product with a particle size below 30µm. It is recommended to press pellets or tablets of the dried biomass, belt drying instead of spray drying of the biomass can also be applied.
- Cell disruption – the microalgae cells have to be disrupted in order to release the lipids/pigments, otherwise the extraction is diffusion controlled and not feasible/possible.

Extraction of crude oil from wet algae biomass with propane (Fig. 5) is a new promising technology. The main advantage of the idea is the fact that the energy-intensive drying of the biomass needed for other types of extraction of algal oils is not necessary. For extraction with propane, the biomass can be concentrated to reach 5-15% of dry weight in the material. Further processes like diafiltration or cell disruption can be applied, when considered necessary. In total, 3.045 kg of wet biomass were processed resulting in production of 8.7 grams crude oil.

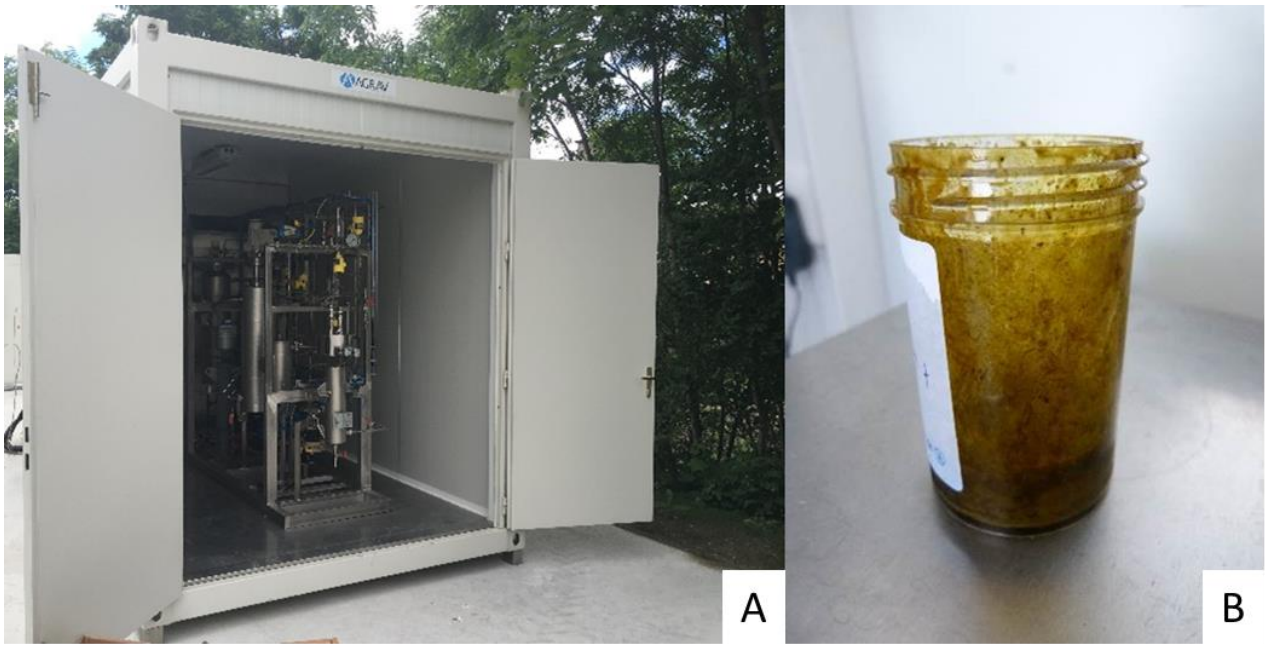

Fig. 5. A – Propane extraction plant for wet algal biomass; B – Crude extract from wet *Prorocentrum* biomass.

The extraction of wet biomass by liquid propane was tested in a pilot plant developed by Natex. The wet biomass concentrated to around 10% of dry mass can be injected into an extraction column on top and is continuously wiped in a thin film on the inner surface of the column. Countercurrently, liquid propane at 22bar and 50°C is fed into the extraction column from bottom. The contact area between the 2 phases is constantly renewed by the wiping system in the column. The propane saturated with extract leaves the extraction column towards the expansion valve and is depressurized into the separator. The crude extract precipitates out of the solution with propane and the propane is recovered in a closed propane circulation system. The crude extract accumulated at the bottom of the separator and can be directly collected (Table 2). The non-soluble part of the biomass – the raffinate, is collected at the bottom of the extraction column.

Table 2. Overview of the biomasses for propane extraction

Strain	Preparation	Dry matter	Extracted wet biomass (g)	Overall yield of crude oil
Chloridella	no cell rupture	8.7%	776.4 g	6.6 w.% of d.w.
Prorocentrum	osmotic shock, diafiltration	10%	2269 g	2.1 w.% of d.w.

The extracts were separated and the crude oil fractions analysed. The composition of the oil phase of the extracts is shown in the Table 3.

Table 3. Examples of fatty acid profiles of the propane extracted microalgae oils

FA (mg/ g oil phase)	Prorocentrum (NXP44-48)	Chloridella (NXP31)
Myristic acid	27.89	52.64
Σ C16	213.75	248.11
Σ C18	219.87	81.45

Stearidonic acid	120.87	n.d.
Σ C20	228.90	215.72
Eicosatetraenoic acid	n.d.	22.35
Eicosapentaenoic acid (EPA)	228.90	193.37
Σ C22	133.62	7.36
Docosahexaenoic acid (DHA)	130.42	n.d.
Σ Others	6.91	10.58
Total	830.94	615.86

It can be concluded, that the target polyunsaturated fatty acids are in considerable amounts in the oil phase of the extracts. The acid values of the oils were again very high, indicating high degree of triglyceride hydrolysis. This alternative technology is capable of extracting the lipid fractions of microalgal origin, but it has to be further developed and optimised.

3.5.3 Impact on other Work Packages

Work developed on this WP provides key inputs for the technology to be considered in WP5 regarding cell disruption and extraction methods. Additionally, oil extracts produced were used for the work developed in WP7.

3.6 WP7 Product Formulation

3.6.1 Work Undertaken

The crude algae oils obtained by NATEX from different strains have been analysed and characterized. The selected strains were *Prorocentrum cassubicum*, *Thalassiosira weissflogii* and *Raphidonema nivale*. Anyhow the crude algae oil of *Prorocentrum cassubicum* has been investigated in more detail since *Prorocentrum cassubicum* was chosen by the consortium to be the main candidate for cultivation, extraction and processing.

3.6.2 Scientific and Technical Results

The characterization of the crude algae oil of *Prorocentrum cassubicum* by GC, UV-VIS and NMR spectroscopy showed no evidence of Glucolipids or Phospholipids being extracted. However a high amount (4-5%) of at least two different chlorophylls was detected. Furthermore the crude oil had an unexpectedly high free fatty acid content of ~50%. Unfortunately, the overall amounts of crude algae oil extracted were too low, hence a model-oil system based on fish-oil has been developed for the investigation and evaluation of the further process steps.

A refining strategy for the purification as well as separation based on selective enzymatic splitting of fatty acids <C20 was developed and elaborated. After an enzyme screening of >10 different enzymes one promising candidate was scaled-up (>2.5 kg) and optimized up to an overall selectivity towards <C20 of >60%. In a pilot-plant scale (>2.5 kg) short-path distillation (SPD) the split <C20 fatty acids were successfully separated from the glyceride fraction. The remaining glycerides were treated again with the enzyme to investigate a potential separation of EPA and DHA. Afterwards a pilot-plant scale short-path distillation (SPD) has been conducted to separate the free fatty acids from the remaining glycerides. A split-ratio of EPA to DHA of 5:1 was achieved.

Since the crude algae oil contains already free fatty acids and the enzymatic splitting generates fractions of free fatty acids a purification method for those value products was developed. All remaining and distilled-off free fatty acids were purified using a urea complexation method. An enrichment of the PUFA fatty acids (i.e. EPA & DHA) of >92% was achieved in only one purification step with an overall yield of >80 %.

3.7 WP8 Demonstration

The amount of algae oil available was unfortunately too low to design a demonstration plant. However, experiments with the model oil (fish-oil) could show the potential of the new enzymatic splitting of PUFA oils combined with the Short-Path-Distillation (SPD).

The original process design was based on Tri-Glycerides (TAG´s) extracted from algae biomass. Therefore, the following process has been developed (Figure 6).

The caustic neutralization step can be skipped, and a direct SPD is conducted. However, the unexpected composition of the algae oil extract with ~50% free fatty acids suddenly lead to a completely new process strategy for the target algae *Prorocentrum cassubicum* at the end of the project timeline (Fig. 7).

The new process strategy was based on the free fatty acids being the target products. Since *Prorocentrum cassubicum* has a very interesting fatty acid profile containing mainly C18:4, EPA and DHA which are all target molecules the direct processing of those fatty acids seems reasonable. The new concept also included a new and innovative product concept of EPA/DHA Magnesium salts.

The commercialization of the PUFA free fatty acids from *Prorocentrum cassubicum* is another strategy. Via the urea-complexation route a fast and easy way to PUFA´s from Prorocentrum cassubicum is available. Figure 8 shows the urea-complexation process.

In this process the purification by urea-complexation is the innovative process step. The SPD to separate PUFA´s from unsaponifiables (i.e. Chlorophyll) might not be necessary if those undesired co-products can be also separated during the urea-complexation.

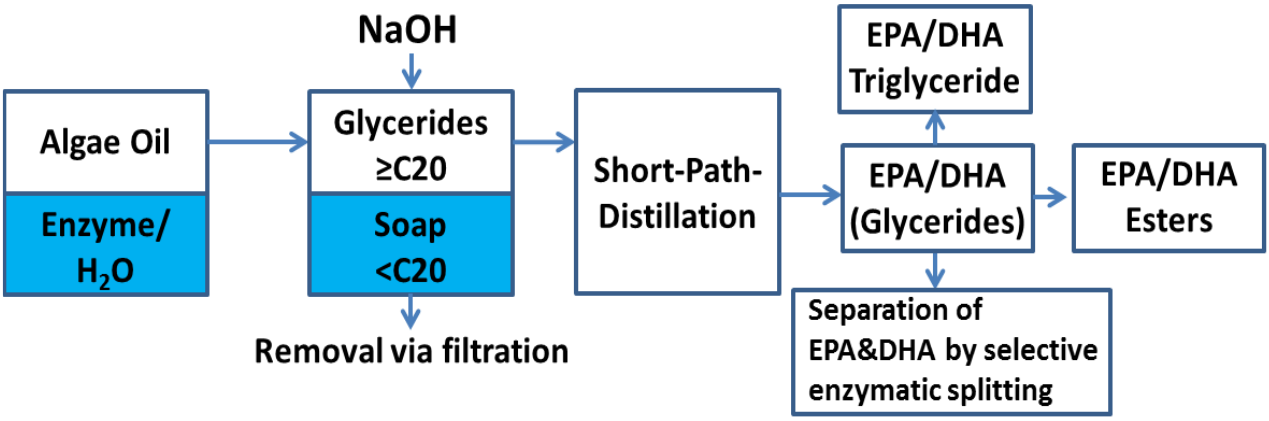

Fig. 6. Simplified Flow-scheme for the enzymatic splitting process of algae oil

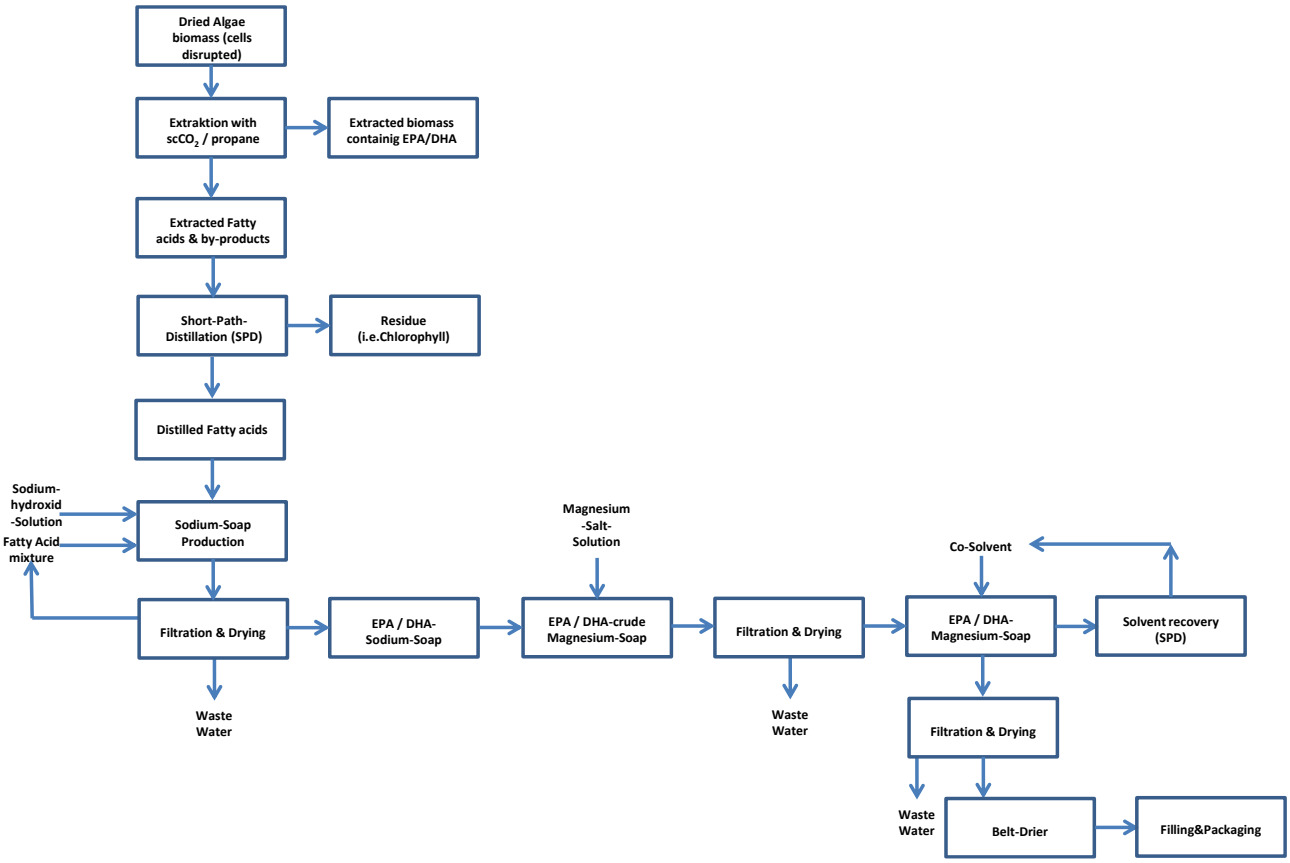

Fig. 7. New process for PUFA-Mg-Salts based on algae oil extracts from *Prorocentrum cassubicum* containing free fatty acids

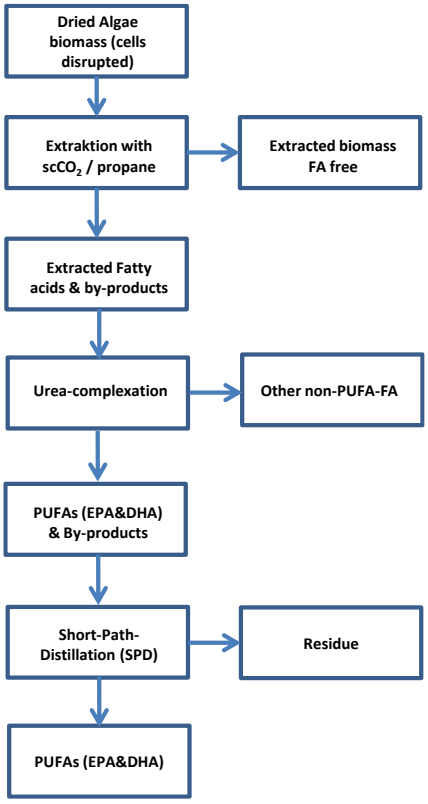

Fig. 8. New process for PUFA-FFA production based on algae oil extracts from *Prorocentrum*

Demonstration cultivation and processing of algae providing stable growth conditions and a reproducible harvesting pattern

The purpose of this task was to demonstrate the feasibility of cultivation and processing of the selected microalgae species in stable growth conditions, which are reproducible in an annual basis in an industrial setting of production. Also, in the scope of the PUFAChain project, besides assuring stable biomass production and harvesting patterns, conservation of the fatty acid profile within controlled acceptable ranges for each microalga is fundamental, in order to provide consistent quality of the biomass produced.

Cultivation of microalgae biomass stabilized

All six strains selected in the scope of PUFAChain project were produced at pilot scale in a semi-continuous regime. These trials were dedicated to optimization and better understanding of growth conditions that lead to higher biomass productivity and omega-3 eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) yields for each microalga.

With the semi-continuous strategy, it was possible to maintain cultures for longer periods in the same photobioreactor. This regime allowed the maintenance of a culture in production in the exponential phase with frequent renewals. Semi-continuous pilot scale cultivations, both in MHT-PBR and UHT-PBR, were accomplished for different strains at A4F Experimental Unit, achieving continuous productions in the same reactor of over 3 consecutive months.

In the scope of PUFAChain project, besides assuring stable biomass production and harvesting patterns, conservation of the fatty acid profile within controlled ranges for each microalga is also fundamental and was achieved. Microalgae fatty acid profiles obtained for laboratory and pilot scale cultures were of extreme relevance and allowed the evaluation of the variability to be expected at industrial scale and consequently its impact in EPA and DHA production yields.

Impact on other Work Packages

Work developed in this WP analyses the data gathered in WP4, establishing the feasibility and reproducibility of the technologies and approaches selected and developed during the project in order to achieve stable biomass production. This analysis validates the use of the data obtained during the project for the work developed WP5 and WP9.

3.8 WP9 Sustainability

An integrated sustainability assessment led by IFEU analyses the sustainability impacts of the newly devised processes. It joins detailed analyses of technological, environmental and socio-economic aspects (by IOI [Reyer et al. 2017], IFEU [Keller et al. 2017a] and WUR [van der Voort et al. 2017], respectively) into an overall picture and derives common conclusions and recommendations [Keller et al. 2017b]. Please refer to these public reports for additional insights and recommendations exceeding this summary, results and methodology.

To this end, algae-based PUFA production was compared to alternatives for meeting additional PUFA demand using fish cuttings, by-catch or by means of fermentation. The aim was to arrive at conclusions on how and under what conditions algae-based biorefineries should be developed in line with the PUFAChain concept. The systems were therefore compared on the basis of scenarios modelling future, industrial-scale, mature processes.

Exemplary quantitative results that lead to the insights summarised below:

Fig 9. shows that environmental impacts determined by life cycle assessment can vary widely depending on boundary conditions and future technological development. These ranges, however, do in big parts not express uncertainty but room for strategic management. We present concrete recommendations how lower ends of these environmental impact ranges can be realised.

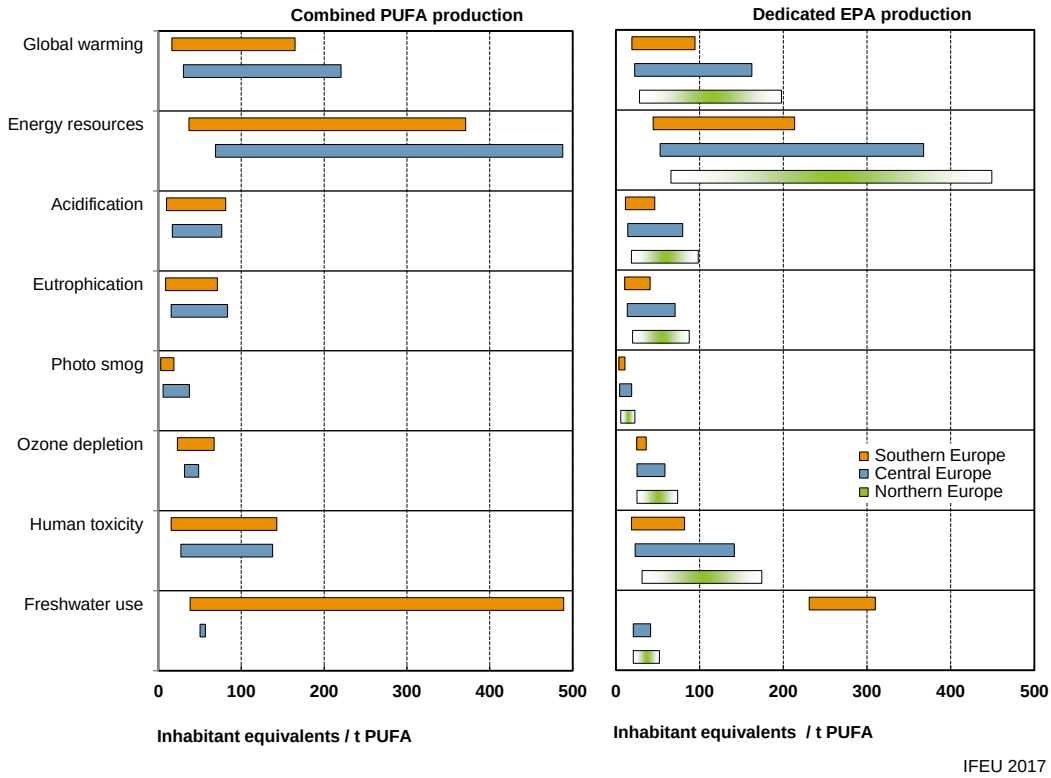

Fig. 9. Ranges of environmental impacts for analysed scenarios of algae-based PUFA production in 2025. Results are expressed in inhabitant equivalents (One inhabitant equivalent corresponds to the annual emissions in that impact category for one average EU inhabitant). Avoided impacts due to use of co-products were credited. Scenarios on combined production of the PUFAs DHA, EPA and SDA use either Prorocentrum or Thalassiosira while dedicated EPA production uses Chloridella in summer and Raphidonema in winter.

The strategic management remark also applies to the economic assessment. The global EPA/DHA consumer market has been growing in recent years and is expected to keep on growing in the future. The production of PUFAs from algae is economically viable. A number of researched scenarios of the micro-economic assessment result in cost prices within the €400 – €1,500 per kg EPA/DHA market price range (Fig. 10).

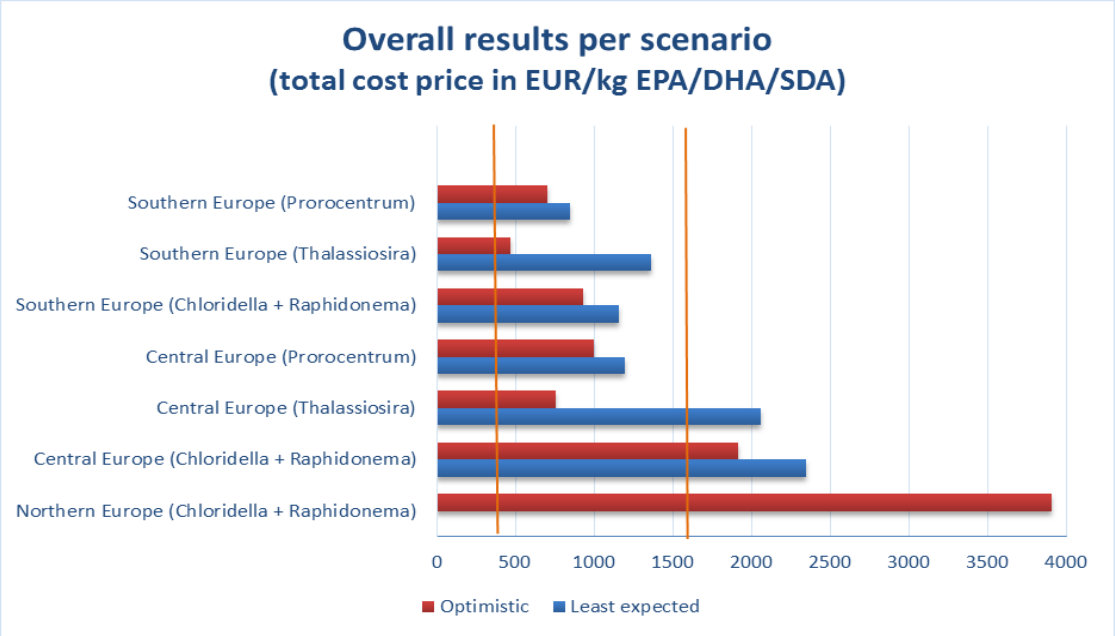

Fig.10. Overall results in cost price EPA/DHA/SDA (€/kg) per region, algae strain for the conservative and optimistic performance scenarios (including market price range).

Key insights are summarised below.

Potentials and need for optimisation. Enormous technological, environmental and socio-economic improvements to algae-based PUFA production were achieved in the course of this project. For example, environmental burdens per tonne of PUFAs can be reduced by up to 80–90% following the analysed scenarios and the costs can be reduced to 50% of the costs of competing PUFA production in the best case. A wide range of technical measures along the entire value-added chain were optimised in detail to achieve this. They include, for example, the use of new algae strains, optimisation of seasonality, site selection criteria, integration of renewable energy generated on site, such as solar electricity, with algae cultivation and many more. In addition, numerous other promising technologies not yet quantitatively modelled in the context of large-scale facilities were investigated in the project. They comprise extraction by means of propane or novel oleochemical purification processes for the extracted oils, for example. It should therefore be anticipated that dynamic technological developments will continue. This means that in the coming years additional breakthroughs, and therefore substantial improvements in technological, environmental and socio-economic sustainability, can be anticipated.

Advantages and disadvantages compared to alternatives. Analysis of the scenarios investigated in this project revealed that, in the coming years, PUFA production employing the PUFAChain concept will probably continue to result in greater global and regional environmental burdens such as acidification, eutrophication, ozone depletion or the use of non-renewable energy resources than competing systems. Impacts on climate change are also greater, but may be indirectly compensated if co-products can displace feeds particularly harmful to the climate from the market. However, it cannot be said with sufficient reliability whether this will actually be the case. From today's perspective, extracting PUFAs from the existing by-products of other processes such as fish cuttings and by-catch therefore tends to be more environmentally friendly.

With regard to other sustainability aspects, benefits result for PUFAs from algae[1] compared to competing systems. They are less dependent on limited resources such as fish cuttings, by-catch or arable land to produce sugar, which is required for fermentation. This can lead to lesser local environmental harm to flora, fauna, soils, etc. Based on the socio-economic evaluation, a PUFAChain could potentially score better than alternatives on employment in Southern Europe and overall on food safety. PUFAs from fish cuttings and by-catch are expected to be less advantageous to health, regarding the risk for contaminants and impurities in natural food chains. In addition, both processes are linked to unsustainable fisheries and therefore will trigger less public commitment. Moreover, no genetically modified organisms are used in algae-based PUFA production, which is often the case in fermentation. Under certain conditions, costs can even be pushed lower than those of the analysed competing products.

Perspectives. Whatever the case, it is better to produce PUFAs such as EPA and DHA, which cannot be extracted from the limited volume of fish cuttings or by-catch, using algae instead of relying on increased fishing to service the growing demand. Here, value-added chains adopting the biorefinery concept developed in this project have enormous potential if the technology and overall utilisation concept continue to be consistently developed. Here, one of the strengths of the concept is that primarily high value–low volume products are addressed and simultaneously large volumes of high quality feeds can be produced. This reduces the danger of future competition for sites and resources such as suitable CO_2 sources. In general, algae harbour great potential as a healthy and sustainable alternative in the food sector. This potential should be developed

[1] Here, 'algae' only refers to photoautotrophic (micro-)organisms, i.e. microorganisms that use light as an energy source. Heterotrophic microorganisms used in competing fermentation processes are often also termed 'heterotrophic algae', which is however in conflict with current scientific consensus.

further, supported by comprehensive sustainability analyses, e.g. by means of integrated life cycle sustainability assessments (ILCSA).

Concrete recommendations to algae community in business and science, to policymakers and to consumers were derived from these conclusions which short, medium and long term action should be taken to improve the sustainability of algae-based PUFA production and which lessons can be learned for algae production and use in general. Please see the “Potential Impact” section of this report for selected key messages and [Keller et al. 2017b] for the complete set of recommendations.

4. Potential Impact and Main Dissemination Activities

Lessons learned from overall PUFAChain

Availability of properly pre-treated algae biomass. The algae biomass availability was crucial for the downstream work packages at the beginning of the project. The properly pre-treated (cell disruption, dewatering, drying) algae biomass was bottleneck for the development of the downstream procedures during the PUFAChain life.

The communication flow between partners. The lack of communication from partners have led to the unexpected delays with deliverables and material and information transfer. The e-mail exchange and internal part of the project web-page were not sufficient to keep information flow between project partners during the project. This can be improved with using existing team communication software. Unfortunately, majority of those tools are cloud based, and industrial partners can have issues with sharing proprietary information through such means.

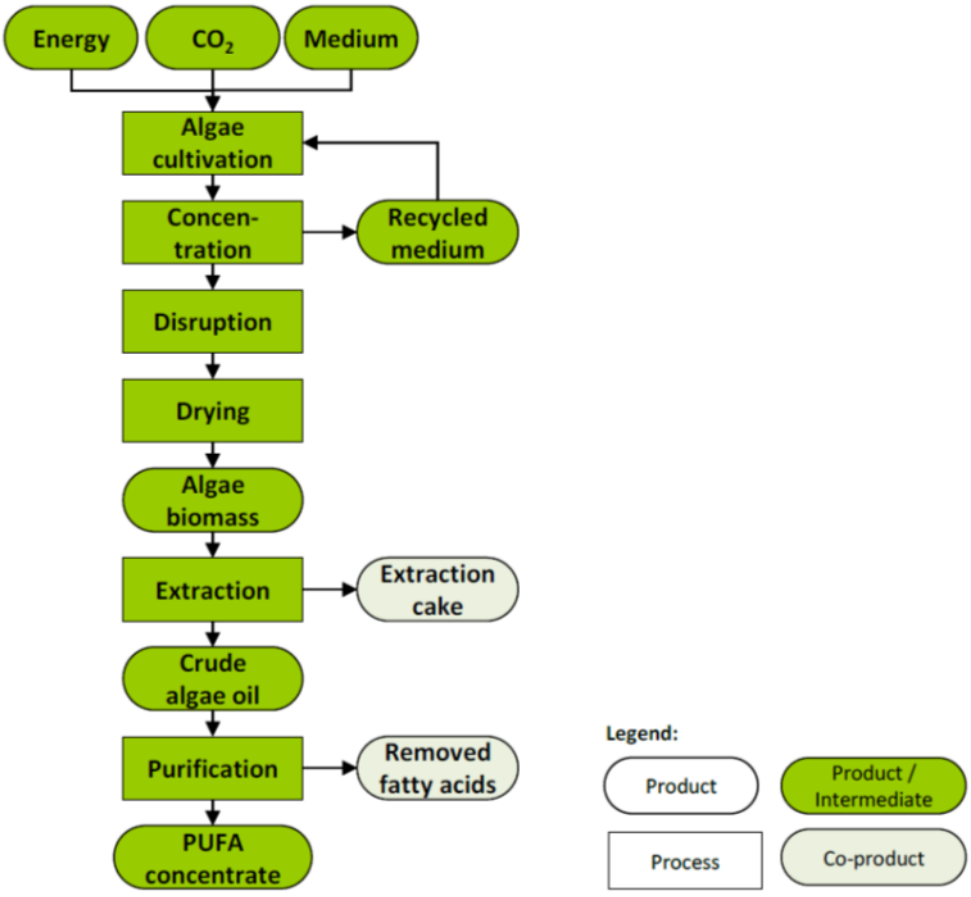

Fig. 11. Overview of life cycle stages of the PUFAChain system.

Target product screening and productive strain selection. The screening process (lipid analysis) was focused on the quantitative (mg / g d.w.) EPA and DHA production by preselected algae strains. Additional analysis of the EPA and DHA distribution among lipid classes (neutral, polar lipids) could give crucial information for the downstream steps of the chain, e.g. expected efficiency of the extraction process (how much of the neutral lipids are present in the algae cell, how much of EPA and DHA are present in the neutral lipids).

Algae cultivation. Selected potential productive algae strain that performed well in small-scale experiments is not suitable for the PBR technology. (1) Low productivity rates (energy input is much higher than output

biomass); (2) low cell densities (more energy spend on harvesting and dewatering); (3) algae cells (without rigid cell wall) are damaged by continuous pumping – no algae growth in PBR; (4) vitamin heterotrophy. Cells of the *Prorocentrum* do not possess external cell wall but it can successfully grow in the PBR (flat panel and tubular PBR).

Algae cultivation protocol should be adapted to the needs of the particular productive algae strain. There is a big potential of the improvement of the PBR technology towards particular producing strain. Adjustments of the PBR technology and cultivation protocol (pumping, medium, PBR architecture, etc.) lead to the improvement of the biomass accumulation of the *Raphidonema*, *Prorocentrum* and *Chloridella* during the PUFAChain project.

Algae biomass concentration. The low cell density algae biomass can cause increase of the energy consumption during harvesting and dewatering stages. Novel strains that were tested during PUFAChain project had lower cell densities in comparison with well-established benchmark strains. However, innovative microfiber filtration reduced energy consumption during harvesting in comparison with centrifugation. It performs well even with comparatively low cell density algae biomass material.

Cell disruption. Cell disruption technologies tested during PUFAChain project were not successful with two EPA producing strains *Nannochloropsis* and *Chloridella*. Unsuccessful cell disruption (less than 50% of cells disrupted) decrease availability of the target products to the extraction. Direct transesterification was tested. However, quality of crude extract (presence of chlorine) make tested method unsuitable alternative. Alternative cell disruption methods (enzymatic cell wall disruption) or extraction strategies needed to be developed.

Drying of the algae biomass. The only available on the industrial scale drying technology – spray drying is not fully compatible with the microalgae material. The size of the resulting particles is small, and can cause unwanted channeling in the extractors. The algae biomass powder should be pelleted after the drying. The spray-drying step can be avoided with the propane liquid extraction method that requires wet algae biomass.

Crude algae oil extraction. Target product availability for the selected extraction methods. Despite the high EPA or DHA content in the algae cells, the majority of targeted PUFAs are present in form of polar lipids. It is known that EPA mostly associated with glycolipids and DHA with phospholipids. So far, there is now industrial scale technology available for the polar lipid extraction from algae material. In the frame of PUFAChain 50 – 70 % of total FA and up to 65% EPA and up to 50 % of DHA were extracted from algae biomass. Nitrogen limitation can induce reallocation of the EPA from polar to neutral lipids. This approach was tested by partner A4F on T. weisflogii CCAP 1085/18. Two phase cultivation was deployed and 50% increase in EPA in the neutral lipids was observed.

Quality of the crude algae oil extract. (1) Presence of the free FA in the crude algae oil extract. High acid values were observed in the crude algae oil. That can mean hydrolysis of the lipids on the different steps of the algae biomass processing. After spray-drying still 7 – 13% of water was in biomass, that can enable lipases activity. Pasteurization of the biomass led to even higher AV. Water is available during pasteurization even longer. (2) Presence of the unsaponifiable materials in the crude algae oil (scCO2 extraction). The conditions of the scCO2 extraction can influence the quantity of the pigments and other substances in the crude algae oil extract. Additional trials using pretreated biomass are needed. (3) Presence of the impurities in the crude algae oil (propane extraction). The additional purification steps are needed to separate algae oils from the water and cell debris.

WP3 Biology

The PUFAChain project had gave additional visibility of the culture collections (SAG and CCCryo) as reliable bioresources. The alternative to the model strains were tested, that support still undiscovered potential of the microalgae in the culture collections. Additionally, collections gain valuable cooperation experience in addressing needs of the industry.

WP4 Bioprocess Engineering

Within the WP4, several strains were cultivated for the first time in outdoor conditions and reached pilot scale cultivation – from 5 L flaks at the lab scale to 1,500 L PBRs at A4F Experimental Unit in Lisbon. For the first time a dinoflagellate was cultivated in tubular PBR on 1.5 m^3 scale. Moreover, a cryophilic strain was cultivated for the first time in outdoor conditions in a PBR on 1.1 m^3 scale during the winter period. The Algal Crop Rotation (ACR) principle was applied to microalgae cultivation and was tested and consolidated on pilot scale for the first time.

In total, 4 production technologies were tested for the pilot scale trials with 6 strains, each of one with their specific cultivation conditions. These strains cultivation was performed in 2 cultivation regimes (batch and semi-continuous) and was optimized within a total of 62 cultivations at pilot scale, lasting a cumulative of 2400 days. At the end, it was possible to scale-up the production to pilot scale level, on a total of 10 m^3, with several technologies, tailored to fit the requisites of each strain.

Within WP4, improvements were introduced to the existing tubular PBRs, namely the coupling of the tubes in only one horizontal plane in order to improve the surface to volume (S/V) ratio of the photobioreactor and therefore potentiate the increase of biomass productivity in the culture. This improvement gave rise to a new type of reactor configuration developed by A4F denominated Unilayer Horizontal Tubular PBR (UHT-PBR). A4F successfully adapted the design, built and tested an optimized system for the PUFAChain project. The performance of a multilayer horizontal tubular PBRs (MHT-PBR) was compared to that of a UHT-PBR at the A4F pilot scale plant in Lisbon. In a MHT-PBR, the photosynthetic area, defined as the projected shadow of the photosynthetic zone of the PBR, varies during the year and during the day, resulting in different productivities. The variation on the photosynthetic area leads to the self-shading of the PBR in the winter or the losing of radiation to the ground in the summer. In a UHT-PBR, the photosynthetic area does not vary during the year and during the day and there is no significant shading between the tubes. Moreover, this design technology significantly lowers capital expenditures (CAPEX), as well as operational expenditures (OPEX), when compared with previously used MHT-PBR.

MAHLE InnoWa developed a new membrane in their laboratory and built up modules for the experimental set-up. This crossflow membrane system is easily scalable to the wished size of installation. Technology parameters for the membrane system were applied during the culture harvesting procedures at A4F Experimental Unit in Lisbon. Consolidated harvesting by membranes, with the versatility and efficiency of culture medium recirculation, was accomplished for the 6 strains cultivated at pilot scale. Moreover, the harvesting system combined with mild methods of cell disruption was developed for 2 marine strains.

WP5 Industrial Production

Initially, a methodology for proposing the most suitable locations for a microalgae biorefinery set-up that can be applied worldwide or in different regions and that must be fine-tuned for each case was developed. Three criteria were selected: (1) the pre-existence of a microalgae farm in the surrounding area so that the industrial facility can be supplied with the required raw material; (2) the existence of knowhow that can be used to create, operate and upgrade the biorefinery; and (3) the proximity to possible consumers of the end product. The chosen five suitable location areas for a PUFA biorefinery in Europe are the region of Ghent (Belgium), the region of Brest (France), the region of Hamburg (Germany), the region of Randstad (Netherlands), and the region of Lisboa-Porto Coastal Axis (Portugal).

Within the WP5, it was developed the pre-engineering project of a Demo scale of 10 ha and an Industrial scale of 100 ha facilities for four different scenarios regarding the two PUFA product of interest (EPA and DHA, and only EPA), being related to specific microalgae strain cultivation, and two geographic locations. The project included all the technologies developed and demonstrated during the PUFAChain project for the microalgae cultivation, biomass harvesting and further processing.

Moreover, a Business Case was consolidated for the industrial biorefinery facilities, with several scenarios reaching interesting profitability (IRR of 17.9 to 39.2%), which will attract investors after the end of the

PUFAChain project. This will promote the implementation of large industrial scale production units of PUFA-rich algal extract, which can lead the way to introduce in the market a more sustainable alternative to fish oil, from autotrophic microalgae cultivation. Partner A4F has already succeeded in promoting the scale-up of the FP7 BIOFAT project, and the FP7 All-gas project has followed the same route. Reports were developed to support the presentation of the Business case of the PUFAChain project to possible investors and a roadmap was designed for the future exploitation of the project. PUFAChain presents a unique investment opportunity for investors interested in nutrient ingredients and pet food specialty ingredients derived from a sustainable feedstock. This investment opportunity's top five value propositions include (1) leading microalgae biorefinery solution provider; (2) value-added product portfolio and a strong market outlook; (3) access to one of the leading networks of algae experts in Europe; (4) flexibility in project configuration; (5) access to low-interest loan guaranteed by the European Investment Bank.

New market opportunities have been identified, in the pet foods and biofertilizer/biostimulant sectors, which can help develop additional markets for microalgal biomass, but also valorise the complete biomass through the biorefinery approach, allowing the algal PUFA oils to gain a more competitive position in the market compared to the traditional fish oil products.

WP6 Downstream

The main exploitable results in WP6 can be summarized as:
Cell disruption methods for specific microalgae strains. The success of the extraction of valuable components from algae cells depends also on the efficiency of the cell disruption. Several different cell disruption methods were tested in scale of several kg's of algae biomass. If any extraction method is going to be exploited, it is essential to add also an effective cell disruption method to the project.

Extraction of neutral lipids from selected dried algae biomass with supercritical CO2. The extraction of neutral lipids from selected dried algae biomass is very interesting process and can be exploited with relatively low technical risk at industrial level.

Extraction of lipids from wet algae biomass with liquid propane. The extraction of lipids from wet algae biomass is a patented process belonging to Natex. The process needs to be further developed and tested on different algal strains. There is already a potential customer interested to exploit this technology.

WP9 Sustainability

The integrated sustainability assessment covered technological, environmental, social and economic aspects. It revealed that most sustainability impacts of industrial scale algae-based PUFA production in 2025 can show enormous variation depending on boundary conditions and future technological development. These ranges of results, however, do in big parts not express uncertainty but room for strategic management by industry, policymakers and research organisations. For example, environmental burdens per tonne of PUFAs can be reduced by up to 80–90% following the analysed scenarios and the costs can be reduced to 50% of the costs of competing PUFA production in the best case. Further improvements by breakthroughs, which cannot be foreseen at this time, seem likely given the current dynamic technological development.

The highlighted optimisation potentials of sustainability impacts, however, can only be reached if decision makers take concrete decisions towards this direction. In such a complex setting as the emergence of a new branch of the bio-based industry, this requires a comprehensive integrated sustainability assessment like the study completed within this project. From its insights, we deduced concrete recommendations how to achieve a reduction of sustainability impacts.

Besides further recommendations in [Keller et al. 2017b], these include in particular:

- Choose the site of a facility carefully because it can crucially influence profitability, environmental and social impacts (to businesses and science).
- Use as much of your own renewable energy, in particular photovoltaics, as possible to run algae cultivation (to businesses and science).

- Optimise algae strain productivity and convert all algae constituents to products (to businesses and science).
- Reduce the energy and water demand for cooling, heating and drying as part of an optimised and integrated concept (to businesses and science).
- Supplying the population with PUFAs such as EPA and DHA can initially be improved by promoting the use of fish residues and by-catch (to policymakers).
- Examine which regulatory requirements can be softened without sacrificing safety or support approvals financially in case of societal benefits (to policymakers).
- If the aim is to establish algae cultivation as a long-term technology, its optimisation must also be correspondingly funded in the long-term (to policymakers).
- Maintain the focus of algae cultivation and use funding programmes on high-value products instead of mass products (to policymakers).
- Only take PUFAs as dietary supplements if this is beneficial for your personal health. (to consumers).
- Be open for new vegetable foodstuffs, e.g. from algae (to consumers).

These and other conclusions and recommendations are aimed at pointing the way to a concrete route for turning algae cultivation, in particular to produce high value food ingredients, into a future component of Europe's bioeconomy.

5.2 Dissemination of the results

The intermediate progress and the results of the PUFAChain project were presented on the number of international and local events (conferences, workshops, etc.) (Table 4). The dissemination activities were targeted towards scientific and industrial communities interested in the microalgae-based biotechnology, as well as wider public. The results we published in two peer review publications, as well as in local papers and Impact journal. The video produced by A4F also generated some interest in the community, gathering over 400 online visualizations in few months.
The results of the PUFAChain project among other topics were presented to the wider public in the frame of excursions through SAG culture collection during “Day of open collections” and “Nacht der Wissens” events, organized in the Georg-August University of Goettingen.
The results of the business plan were disseminated in several international conferences, in Europe and in the USA, major occasions of meeting and business for the consortium and the investors.

Table 4. Summary of the dissemination activities during life span of the PUFAChain project

Type of activity	Number
Peer-review publications	2
Popular	1
Oral presentation to a scientific event	18
Articles published in the popular press	3
Oral presentation to a wider public	4

Use and dissemination of foreground

Section A

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided to this publication?
1	Integrated life cycle sustainability assessment – A practical approach applied to biorefineries	Heiko K.	Applied Energy	Vol. 154	Elsevier BV	Netherlands	2015	1072-1081	https://doi.org/10.1016/j.apenergy.2015.01.095	Yes
2										

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).
[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities[4]	Main leader	Title	Date/Period	Place	Type of audience[5]	Size of audience	Countries addressed
1	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	PUFAChain: The value chain from microalgal diversity to PUFAs: technological, environmental and integrated sustainability assessments	02/04/2014	2nd European Workshop Life Cycle Analysis of Algal based Biofuels & Biomaterials, Brussels	Scientific community (higher education, Research)	50	Belgium, Germany
2	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	The PUFAChain project and assessing species distinctions among EPA producing eustigmatophytes	07/03/2016	16. Wissenschaftliche Tagung der Sektion Phykologie, Leipzig, Germany	Scientific community (higher education, Research)	70	Germany

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

3	Articles published in the popular press	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	New European Project: PUFAChain	23/05/2014	Goettingen International Newsletter	Scientific community (higher education, Research) - Civil society		Germany
4	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	The PUFAChain project: a value chain from algal biomass to lipid-based products	03/06/2014	7. Bundesalgenstammtisch, Koethen, Germany	Scientific community (higher education, Research) - Industry	70	Germany
5	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	How to extend an LCA of algal biomass pathways to a conclusive sustainability analysis	16/06/2014	4th International Conference on Algal Biomass, Biofuels & Biomaterials, Santa Fe, USA	Scientific community (higher education, Research) - Industry		USA
6	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	Conclusive Sustainability Assessment of Algal Biomass Pathways through considerable Extension of LCA Application	25/06/2014	22nd EU BC&E Algae event, Hamburg, Germany	Scientific community (higher education, Research) - Industry		Germany
7	Articles published in the popular press	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	Von den Algen wird noch Großes erwartet	22/10/2014	Rhein-Neckar-Zeitung	Civil society		Germany

8	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	How green are algae? Assessing sustainable development of algae production	30/10/2014	1st Biooekonomie-Kongress Baden-Wuerttemberg, Germany	Scientific community (higher education, Research) - Industry		Germany
9	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	PUFAChain - a value chain from algal biomass to lipid-based products	02/12/2014	1st EC Algae Contractors' Conference, Florence, Italy	Scientific community (higher education, Research) - Industry		Germany, Italy, Portugal, Spain, Great Britan, Netherlands
10	Articles published in the popular press	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	Bioenergie und Biomaterialien aus Algen	22/01/2015	Biobased Future, 3rd Issue 2015	Scientific community (higher education, Research) - Industry		Germany
11	Oral presentation to a wider public	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	The PUFAChain project and assessing species distinctions among EPA producing eustigmatophytes	06/03/2015	16. Wissenschaftliche Tagung der Sektion Phykologie, Leipzig, Deutschland	Scientific community (higher education, Research)	80	Germany
12	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	Overview, status and prospects on LCA for algae products ? Keynote speech	11/05/2015	3rd European Workshop "Life Cycle Analysis of Algal based Biofuels and Biomaterials", Brussels	Scientific community (higher education, Research) - Industry		Belgium, Germany

13	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	PUFAChain - a value chain from algal biomass to lipid-based products	18/06/2015	Congress "Eco-innovations from biomass", Papenburg, Germany	Scientific community (higher education, Research) - Industry		Germany
14	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	PUFAChain - a value chain following the biorefinery concept from algal biomass to lipid-based products	19/06/2015	ACHEMA 2015, Frankfurt, Germany	Scientific community (higher education, Research) - Industry		various countries
15	Oral presentation to a wider public	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	The European PUFAChain project (FP7) - a value chain from algal biomass to lipid-based products	28/08/2015	6th European Phycological Congress, London, England	Scientific community (higher education, Research)	450	various countries
16	Oral presentation to a scientific event	FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V	The Culture Collection of Cryophilic Algae (CCCryo): A biobank connecting field work with industrial photobioreactors. - Proceedings of the ESBB Annual Conference, London	30/09/2015	ESBB Annual Conference 2015, London, England	Scientific community (higher education, Research) - Industry		various countries

17	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	PUFAChain - a value chain from algal biomass to lipid-based products	02/12/2015	Algae Europe 2015, Lisbon, Portugal	Scientific community (higher education, Research) - Industry		various countries
18	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	LCA of algal products: state of the art and perspectives	13/12/2016	"AlgaEurope 2016", Madrid, Spain	Scientific community (higher education, Research) - Industry		various countries
19	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	LCA of algae based biorefineries: actual state of the art worldwide and perspectives	07/07/2017	13th International Conference on Renewable Resources and Biorefineries, Wroclaw, Poland	Scientific community (higher education, Research) - Industry		various countries
20	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	The PUFAChain project: a value chain from algal biomass to lipid-based products	16/08/2017	The Eleventh International Phycological Congress, Szczecin, Poland	Scientific community (higher education, Research) - Industry	450	Germany, Great Britan, Poland, USA, Russia, Ukraine, Spain, Portugal, South Korea
21	Oral presentation to a scientific event	GEORG-AUGUST-UNIVERSITAET GOETTINGEN STIFTUNG OEFFENTLICHEN RECHTS	EU FP7 project PUFAChain - bringing links of value chain together	17/10/2017	"Algae Biorefineries for Europe", Brussel, Belgium	Scientific community (higher education, Research) - Industry - Policy makers	70	Germany, Spain, Great Britan, Israel, Portugal, Netherlands

22	Oral presentation to a scientific event	ifeu - Institut fuer Energie- und Umweltforschung Heidelberg Gmbh	Sustainability of algae cultivation and use: conclusions and recommendations	17/10/2017	Algae Biorefineries for Europe 2017, Brussels, Belgium	Scientific community (higher education, Research) - Policy makers		various countries
23	Oral presentation to a scientific event	FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V	The top 5 options to make algae products more sustainable: lessons learnt from recently completed studies in Europe	29/10/2017	Algae Biomass Summit 2017, Salt Lake City, USA	Scientific community (higher education, Research) - Industry		various countries

Section B Part B1

LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[6]:	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)

[6] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Type of Exploitable Foreground[7]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[8]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	Ex: New supercond uctive Nb-Ti alloy			*MRI equipment*	*1. Medical 2. Industrial inspection*	*2008 2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[8] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html