Publishable Summary

Summary description of 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 6 companies and 3 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, acryonyms 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, CO_2 supply, disposal of O_2, 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 upscaling from laboratory production tests (e.g. 10 L) to pilot scale cultivation (up to 1 m^3, 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 CO_2 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.

Description of work performed and main results so far

More than 100 algal samples/strains from both bio-resource providers were analysed for their PUFA profiles. Specifications such as a general good growth and in particular a significant content of EPA and DHA with the preferably selective production of one of those, coupled with no or at least minimal ARA content were already specified during the 1st reporting period. Because of an almost 1:1 relation of EPA/DHA, both in significant amounts while no ARA is detectable, the first choice from all strains tested up to now is still the dinoflagellate *Prorocentrum cassubicum* SAG 40.80. It has been successfully cultivated by partner A4F up to a pilot scale of 1.1 m^3, despite its the fragile nature. The screening also identified two strains from UGOE producing EPA in high amount and purity: *Chloridella simplex* SAG 51.91 and *Nannochloropsis gaditana* SAG 2.99. All samples from the collection of arctic microalgae from partner Fraunhofer either exhibited lower lipid contents or had unfavourable lipid profiles. Based on the initial list of potential algal strains (D3.1) a selection of 22 strains after PUFA/metabolite testing was made which will be considered for further analyses (D3.1). The selection includes the nine "best" EPA only producing strains, i.e. nine Stramenopile algae from the UGOE growth experiments. 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. To assist successful upscaling, miniaturized cultures in a cross gradient table with light and temperature gradients to evaluate the optimum temperature and light intensity and range of tolerance to those factors have been introduced.

Seven of the selected strains were characterized in terms of productivity and fatty acid content at the lab scale (5 L flasks) and four groups of strains with significant potential were revealed. On the basis of those results so far four of the most promising strains were successfully upscaled to pilot scale, i.e. 1,100 L in a tubular photobioreactor (PBR) under outdoor conditionswithin a green house at Lisbon, Portugal. Typical productivity values for outdoor cultures were recorded ranging from 11 g/m^2/day to 15 g/m^2/day and lipid profiles under those culture conditions were analysed. For downstream processing, two testing series were carried out using a novel microfiltration system already commissioned at the PBR pilot unit and *Phaeodactylum tricornutum* SAG 1090-6 as a test and reference strain. For this strain dried biomass was produced and subjected to crude oil extraction by means of supercritical CO_2. These experiments underlined the need of a successful cell disruption before lipid extraction. For the extraction of wet algal biomass by liquid propane, a plant is still under construction.

To deliver an integrated assessment of the sustainability of the entire value chains investigated in PUFAChain technological, environmental, economic and social aspects are taken into account. An

interim report of definitions, settings and system descriptions (D9.1) has been submitted. Key achievements were the refinement of and agreement on common definitions, settings and system descriptions. A life cycle assessment (LCA) tool has been set up and covers full life cycle comparison of PUFAChain to other reference systems. An “Internal SWOT workshop” has been performed with all project partners and it identified issues relevant for the socio-economic assessment.

The existing communication procedures (internal project area; project handbook; mailing lists) have been maintained and kept up-to-date. Posters and documents illustrating the overall project content and collaboration are available from the PUFAChain website, www.pufachain.eu. In addition, 3 project newsletters have been issued through the website so far. In the meantime, the awareness of PUFAChain has been significantly increased as the project has been presented on various important events all over Europe and even beyond. The second General Assembly Meeting of “PUFAChain” was held October 6-8 in Ternitz, Austria.

Expected final results and potential impacts

The objective is to develop a sustainable source of high-purified omega-3 fatty acids (DHA /EPA) as building blocks in modern oleo chemistry to gain high value products for nutrition and pharmaceutical applications. DHA and EPA are widely used in high value products for nutrition and pharmaceutical applications. The aim is to develop the suitable preparation of these high-purified algae oils as well as the exploitation of the side products and residues. Accompanying high-value substances may be colorant antioxidants, proteins and enzymes for pharmaceuticals and polysaccharides as surface active compounds which will be considered for exploitation as well. 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 while the project is strongly industry driven. A cultivation process adapted to the needs of the end-users specification and the selected strains will be installed.

At the downstream processing three stages will be adapted to the viable and efficient treatment of the cultured algal biomass and combined in a suitable process chain in order to obtain high purified omega-3 fatty acids in optimal condition. The stages “Cell Disruption”, “Crude Oil Extraction” (with evaluation of supercritical and liquid solvent extractions) and “Separation of defined molecule classes” will be further investigated. This follows an integrated membrane filtration for harvesting which will be developed to provide the possibility to remove bacterial loads and cell fragments for efficient water recycling and reuse of the nutrients. To demonstrate the whole process a demonstration plant will be built at the end-user’s (partner CRM) site combining all necessary steps and demonstrating the viability of all technical interfaces.

A comprehensive and holistic sustainability approach, addressing environmental, economic and social aspects, will complement the scientific and commercial advances on each value-adding stage. The economic, environmental and social viability will be monitored and evaluated by a dedicated approach conducted by two independent institutes which are partners in the project. They will also actively provide other participants with advice during the realisation of the project so that the early implementation of economic, social and environmental needs will be ensured.