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, 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, CO_2 supply, disposal of O_2, distribution of light intensity in the photobioreactor and photosynthetic efficiency) will 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 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 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

To optimize PUFA yields in the previously selected 14 algal strains optimal culture conditions were revealed. Temperature was found the major factor to directly influence EPA content. A cryophilic green algal strain, *Raphidonema*, with considerable EPA values even at 4°C and being well adapted to low light conditions, was identified thus promising to enable the ACR principle and pilot plant trials in a horizontal tubular photobioreactor under winter conditions in Southern Europe further supported the results. Variations nitrogen/phosphorus ratio, addition of the vitamins and CO_2 aeration influenced both, biomass and target substances production as revealed by small-scale *Kniese* airlift system photobioreactors. Two groups, i.e. with a lower and a higher temperature range, were identified among the tested strains using in a temperature/light cross gradient of miniaturized cultures. The effect of CO_2 addition may be related to phosphate concentrations of the culture media. The addition of the vitamin B12 did not have positive effect on biomass accumulation or EPA/DHA content in any of the tested strains, but biotin may be required to sustain growth of *Prorocentrum*. As potentially exploitable side-products tocopherol in *Prorocentrum* and, as expected, fucoxanthin in the two tested diatom strains were found.

Two appropriate main production scenarios were identified, (1) EPA and DHA production with *Prorocentrum* (plus *Thalassiosira* as established benchmark strain for reference) and (2) Algal Crop Rotation for EPA with mesophilic *Chloridella* (plus *Nannochloropsis* as benchmark strain) and cryophilic *Raphidonema*. Osmotic shock through diafiltration was found a highly efficient method for cell disruption of *Prorocentrum* and, similarly, for *Thalassiosira* as well. Cell disruption may be an essential prerequisite for successful oil extraction as it was shown in the previous reporting periods. However, cell disruption has been unsuccessful for *Chloridella* and *Nannochloropsis* so far, but direct transesterification with an acid catalyst may be promising for both strains. Biomass concentration was achieved through a novel crossflow microfiltration membrane system, including optimization of hollow fibre membrane material. Supercritical CO_2 extraction experiments with dried algal biomass were successful with *Prorocentrum* and two tested diatom reference strains following cell disruption by osmotic shock. For the subsequent chemical characterization of the final crude algal oil (including trials to separate DHA and EPA from each other) enzymatic catalysis for lipid oil splitting could be further evaluated. More extraction experiments (incl. liquid propane for the wet algal biomass extraction) and further algal oil characterization will be performed as soon as new algal biomass will be available early 2017.

A crucial decision that was taken on project level to narrow down the selection of algal strains. This has an impact on seasonality and geographical aspects of algae cultivation, most favorable products and product portfolios, as well as on harvesting, algae disruption and downstream processing. Furthermore, this now allows the sustainability assessment to transition from an interim phase of validating models and generating generic insights to more project-specific fine-tuning, modelling and

results. Good data quality was achieved for cultivation of the newly selected algae strains. Consequences of the decisions on algae strain selection for the whole life cycles of the assessed scenarios were evaluated in a technological assessment. The most important insight was that not only algae cultivation and disruption was substantially affected, but almost all following life cycle stages. A macro-economic analysis was conducted with an online survey of market information for the main products of the PUFAChain and the identification of competitors. Concurrently, the databases for project-relevant target groups established in the first project year have been significantly extended. The project website, www.pufachain.eu, has continuously been updated and a total of six newsletters have been issued until now. The third General Assembly Meeting of "PUFAChain" was held October 4-6 2016 in Lelystad, Netherlands.

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-user's 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 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.