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EXECUTIVE SUMMARY

Objectives

The objective of this report is to present the findings of the final Life Cycle Assessment (LCA) of the current and future production practices of *Saccharina latissima* and *Ulva* spp. and its processing into products.

This assessment is based on site-specific data provided by the producers, namely Seaweed Solutions for *Saccharina latissima*, ALGAplus for *Ulva rigida,* Algaia for the current processing of wild *Laminaria digitata* (as a proxy for *Saccharina latissima*) and the future processing of *Saccharina latissima* from Seaweed Solutions, and Olmix for the current processing of wild *Ulva* spp. and the future processing of *Ulva rigida* from ALGAplus.

The report includes an analysis of the environmental impacts of processing methods for new seaweed applications, currently operating at lab scale, and identification of environmental hotspots within the steps and focal points for scale-up. The processing methods are the treatment of *Saccharina latissima* and *Ulva rigida* with two enzymes and the supercritical carbon dioxide extraction of carotenoids among which fucoxanthin from *Saccharina latissima.*

Rationale

This report is developed under task 5.4 that aims at evaluating the environmental performance of seaweed products by means of LCA, which is used in GENIALG as an instrument of refinement and optimization of both aquaculture and biorefinery. LCA is a standardized methodology that compiles and evaluates the environmental aspects and impacts of a product throughout its life cycle.

Seaweed production and current processing

The current and future cultivation of *Saccharina latissima* by Seaweed Solutions takes place in off-shore farms in Norway and includes the steps of collection of reproductive material, hatchery, transport of sporelings from the hatchery to the port, off-shore farm, and transport to pre-processing. *Ulva rigida* is currently cultivated by ALGAplus in a land-based Integrated Multi-Trophic Aquaculture system in Portugal. The current processing of wild *Laminaria digitata* and future processing of *Saccharina latissima* from Seaweed Solutions by Algaia in France gives origin to alginate products and comprises the steps of transport to the processing factory and processing. The current processing of wild *Ulva* spp. by Olmix in France gives origin to the product Aralgae and comprises the steps of wild seaweed harvesting, transport to the processing factory and cleaning; extraction; and solid fraction processing. The future scenario of *Ulva rigida* from ALGAplus processing at Olmix facilities includes the steps of transport, extraction and solid fraction processing.

Results

The results of this final LCA highlight the main environmental hotspots of the seven systems analysed. The largest environmental impacts in *Saccharina latissima* cultivation (current and future) derive mainly from the use of diesel, but the use of steel anchor chains and the nitrogen emissions to water are also relevant. The impacts from the current *Ulva rigida* cultivation are almost exclusively associated with the use of electricity. In the current processing of *Laminaria digitata*, the largest contributions come from the consumption of natural gas and electricity during alginate production, as well as from

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emissions to water. In the future processing of *Saccharina latissima* from SES at Algaia facilities, these contributions remain the most important except for ozone formation and terrestrial acidification, for which transport is the main contributor. In the current processing of *Ulva* spp. and the future processing of *Ulva rigida* from ALGAplus at Olmix facilities, the largest contributions come from the use of propane gas and electricity, and also from emissions to water.

Regarding the comparison of current and future practices, the future *Saccharina* cultivation has a better environmental performance than the current one. On the contrary, as a result of the greater transport distance, the future processing at Algaia facilities will lead to larger impacts than the current processing (although in some impact categories the increase is negligible), and the future processing at Olmix facilities is environmentally worse than the current processing in four of the seven impacts analysed and better for freshwater and marine eutrophication and mineral resource scarcity.

Future seaweed processing

The processing of seaweed by treatment with two enzymes was assessed. From each treatment, a solid and liquid fraction result with applications of interest to the GeniAlg project partners. Sequential single enzyme treatments were assessed for *Saccharina latissima*, for *Ulva rigida* and in an optimized lab scenario for an averaged seaweed. The optimized scenario for an averaged seaweed was also assessed on a higher scale level, following the lab incubation time, a shortened incubation time and a simultaneous treatment with two enzymes.

It was found that heating and enzymes contribute most to most environmental impacts. Upon optimization and scale-up, heating requirements are significantly reduced thanks to an increased concentration. Incubation temperature and enzyme consumption will change in practice and will affect the environmental impacts for both lab and scale-up scenarios. For all scenarios, the overall variation in the environmental impacts is substantial. Despite this, the trends from the results are sufficiently robust to derive some recommendations. The scale up should focus on yields, concentration and temperature differential, as this will reduce the environmental impact strongly. Knowledge about the applications of the different fractions from treatment will aid interpretation and optimization.

The processing of seaweed by supercritical carbon dioxide extraction was also assessed. The three scenarios focused on different drying methods. The energy required differs strongly for these methods. These differences determine scenario differences and hotspots. If drying energy decreases, the electricity required for pressurizing the carbon dioxide for the extraction becomes a hotspot. The drying and pressurizing determine the impact across all environmental impacts. The overall variability analysis shows the trends are robust despite the limitations. It is expected scale-up will not change the trends in the results, but a trade-off between energy efficiency and mild conditions should be found for the drying method.

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1 INTRODUCTION

The GENIALG project aims to boost the Blue Biotechnology Economy by increasing the production and sustainable exploitation of the brown algae *Saccharina latissima* and the green algae *Ulva* spp. The Work Package 5 (WP5) assesses the economic feasibility and environmental sustainability of cultivating and refining this seaweed biomass in multiple use demanded products of marine renewable origin. In particular, task 5.4 aims at evaluating the environmental performance of these products by means of Life Cycle Assessment (LCA). This tool is used as an instrument of refinement and optimization of both aquaculture in WP3 and biorefinery in WP4.

In GENIALG, many processing options were piloted for the two seaweed species of focus with the aim of producing useful products for five application areas: texturants and other food applications, neutraceuticals and soil improvements, cosmetics, pharmaceuticals and bioplastics. Since it is expected these products contribute to improved sustainability in these areas, their environmental impact should be evaluated. In the first two application areas, pre-existing products are produced at an industrial level and the pre-existing products will be studied in order to map the environmental hotspots and give a perspective on the environmental performance of improved processing (future scenarios). This assessment is based on site-specific data provided by the producers, namely Seaweed Solutions for cultivation of *Saccharina latissima*, ALGAplus for cultivation of *Ulva rigida,* Algaia for the current processing of wild *Laminaria digitata* (as a proxy, because currently Algaia doesn´t use *Saccharina latissima*) and the future processing of *Saccharina latissima* from SES, and Olmix for the current processing of wild *Ulva* spp. and the future processing of *Ulva rigida* from ALGAplus. The results from this LCA are relevant to highlight environmental hotspots, to allow adjustment of processes, and to anticipate if future scenarios will lead to environmental gains.

For the latter three application areas mentioned above, several processing options are piloted, with different purposes and different technological maturities. The following treatments are considered:

- Enzymatic treatments of both seaweeds yield interesting active ingredients for cosmetics, but for neutraceutical this may also give interesting new products or product improvements (BDC, 2021).
- Different extractions from *Saccharina latissima* yield potential active ingredients, among others for pharmaceutical applications. First, fucoxanthin can be extracted along with other carotenoids with supercritical carbon dioxide extraction (SCE). Another SCE step, perhaps assisted with microwave radiation, could yield mannitol. If microwave assistance is avoided, an acid extraction that follows will yield fucoidan. The alginates can be extracted with a conventional industrial method afterwards (University of York, 2021).
- Various methods could be employed to obtain acrylic acid from *Ulva rigida*, such as sonication, perhaps on seaweed that has been stressed during growth or pretreated with enzymes. However, because of low yields, the metabolic pathways from the seaweed to produce acrylic acid might be better developed in a platform organism such as cyanobacteria (BDC, 2021, University of York, 2021).

The enzymatic treatments can be combined with the extractions or with the industrial production of neutraceuticals or texturants. By leaving out steps from the extraction series, different combinations of treatments can be envisioned. The environmental impact strongly depends on the combination of processing steps, and how these will be combined is unknown. The environmental impact of double enzymatic treatments and of the fucoxanthin extractions has been mapped with a LCA focussing on

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the processing step only, so that the environmental hotspots can mapped within the step, and the environmental information can be used for further integration of various seaweed processing steps into a biorefinery.

This report is organized in 4 chapters. The first one presents the rationale and the objective of this report. Chapter 2 describes the LCA methodology applied and indicates the impact categories and method considered for the impact assessment. Chapter 3 describes the LCA of current situation and future scenarios of cultivation and processing of the different seaweeds, including the results obtained and conclusions. Chapter 4 describes the LCA of the new seaweed applications.

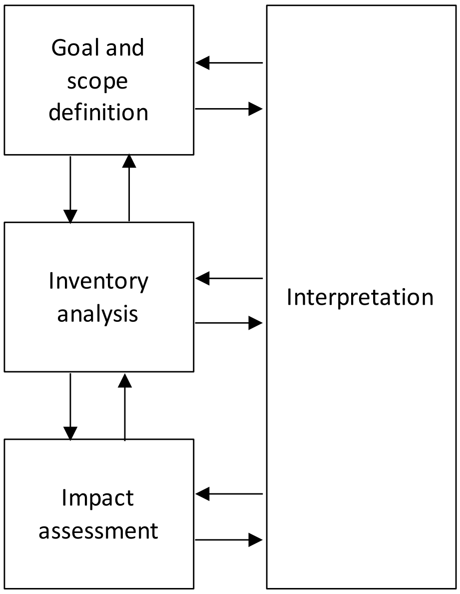
2 General method

2.1 Life Cycle Assessment

LCA is a standardized methodology that compiles and evaluates the environmental aspects and impacts of a product throughout its life cycle (ISO, 2006). The life cycle may comprise extraction and processing of raw materials, production, use, and eventual reuse, recycling or disposal at end of life. LCA is generally considered the most suitable method for assessing the environmental impacts of products since it addresses both the entire life cycle of a product and the full spectrum of environmental impacts (Zamagni et al., 2009). The advantages of a life cycle approach to address sustainability are well known and relate with avoidance of shifting problems, for example, from one life cycle stage to another, from one geographic area to another and from one environmental medium (e.g. air) to another (e.g. water).

LCA is structured in four phases (ISO, 2006), as shown in Figure 1: (1) goal and scope definition, (2) inventory analysis, (3) impact assessment, and (4) interpretation. This framework allows for iterative procedures among phases. As the assessment unfolds, data limitations and new insights or stakeholder views can lead to a redefinition of the study focus, goals or methods.

Figure 1: Structure of LCA



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The goal and scope definition mainly includes aspects such as:

- definition of the goal, including, the intended application, the reasons for carrying out the study and the intended audience;
- definition of the system boundaries, i.e., the unit processes along the life cycle to be included in the analysis;
- definition of the functional unit, which is the reference unit in relation to which the inventory and impact indicators are expressed;
- definition of the initial data quality requirements;
- selection of impact categories and respective methods for impact quantification.

The inventory analysis involves data collection for the processes previously identified within the system boundaries. These data comprise environmental aspects such as consumption of raw and ancillary materials and energy, as well as emissions to air, water and soil and solid waste generation. The inventory analysis also includes calculation procedures so that the data collected for each process are summed up and related to the functional unit.

During the impact assessment, the inventory data are processed in terms of their environmental impacts previously selected in the scope. This phase encompasses three mandatory elements, as follows:

- selection of impact categories, category indicators, and characterization models; there are currently several models available that include impact categories such as climate change, acidification, eutrophication, and resource depletion, among others;
- classification, where the inventory parameters are assigned to specific impact categories;
- characterisation, where equivalency between inventory parameters within each impact category is done considering the cause-effect chain (environmental mechanisms) by means of so-called characterisation factors, resulting in an indicator for each impact category.

The impact assessment phase may also include the following optional elements:

- normalisation, where the results from the characterisation are compared to a reference situation that could be the total impacts of a country or a region, for each impact category;
- grouping, which consists of sorting and possibly ranking the impact categories;
- weighting, where the indicator results of the different environmental impacts are weighted relative to each other by using numerical factors based on value choices. Weighting may include aggregation of the weighted results into a single score.

Finally, the interpretation consists in the analysis of the results obtained both at the inventory and the impact assessment phases. This analysis allows the detection of the main impacts, their sources and improvement opportunities. In addition, the quantitative results obtained should be critically analysed and verified in terms of consistency, completeness, coherence, and agreement with the initial expectations.

2.2 Impact assessment

The impact assessment includes the mandatory elements up to the characterisation. The method selected was the Hierarchist version of ReCiPe2016 at midpoint characterization level (Huijbregts et al., 2017). From this method, a number of environmental impacts was selected. These environmental

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impacts are common issues which are relatively robustly characterized with the method. Therefore, the following impact categories were considered: global warming (GW), ozone formation - human health (OF-HH), ozone formation - terrestrial ecosystems (OF-TE), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), mineral resource scarcity (MRS), and fossil resource scarcity (FRS). The results obtained for the category OF-TE are not presented in Part I because they are similar to those obtained for OF-HH. In a more agricultural context, land use and water use would be relevant as well, but were not addressed in this study. The optional elements of normalization, grouping and weighting were not applied because they are not necessary to achieve the goal defined for this study.

3 PART I: LCA OF CURRENT SITUATION AND FUTURE SCENARIOS

3.1 Methodology

3.1.1 *Goal and scope definition*

3.1.1.1 Goal

The goal of this study is to evaluate the environmental performance of the current practices of: (1) *Saccharina latissima* cultivation in Norway, (2) *Ulva rigida* cultivation in Portugal, (3) wild *Laminaria digitata* processing in France into alginates and (4) wild *Ulva* spp. processing in France into Aralgae (a solid clay-based product), using site-specific data provided by the producer companies Seaweed Solutions, ALGAplus, Algaia and Olmix, respectively; and the future scenarios of (5) *Saccharina latissima* cultivation at Seaweed Solutions, (6) processing of *Saccharina latissima* from Seaweed Solutions at Algaia facilities and (7) processing of *Ulva rigida* from ALGAplus at Olmix facilities.

The main purpose is to quantify the life cycle impacts of the current and future practices and to identify the processes with the largest impacts, as well as to compare the current and future scenarios. The intended audience are the partners of the GENIALG project and other interested parties in the topic.

3.1.1.2 System boundaries

The system boundaries for the current and future cultivation of *Saccharina latissima* include the steps of collection of reproductive material, hatchery, transport of sporelings from the hatchery to the port, off-shore farm, and transport to pre-processing (Figure 2). Besides, carbon dioxide (CO_2) uptake from the atmosphere and nitrogen (N) uptake from seawater (bioremediation) during seaweed growth were also considered.

For the current cultivation of *Ulva rigida*, the boundaries include the process of cultivation in ponds under Integrated Multi-Trophic Aquaculture (IMTA) (Figure 3), as well as pre-processing by washing and centrifugation. The fish ponds of the IMTA system were left outside the boundaries as the corresponding environmental burdens are not affected by the seaweed production activity. The uptake of CO_2 and N during seaweed growth was also considered.

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The system boundaries of current *Laminaria digitata* processing and future *Saccharina latissima* processing into alginates (Figure 4) comprise the steps of transport to the processing factory and processing at Algaia facilities.

The system boundaries of current *Ulva* spp. processing into Aralgae (Figure 5) comprise the steps of wild seaweed harvesting, transport to the processing factory and cleaning, extraction, and solid fraction processing. For the future *Ulva rigida* processing (Figure 5), the steps considered are transport from ALGAplus to Olmix facilities, extraction, and solid fraction processing.

In all systems, the production of energy, fuels, water and materials consumed in all the processes was also considered. However, the production of the clay integrated in Aralgae produced by Olmix is excluded from the boundaries both in current and future scenarios. The production of capital goods (buildings, machinery and equipment) was excluded. However, in the *Saccharina latissima* system, the replacement of the infrastructure of the farm was included as data were available for the flows of materials replaced each year.

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Figure 2: System boundaries for the current and future cultivation of Saccharina latissima.

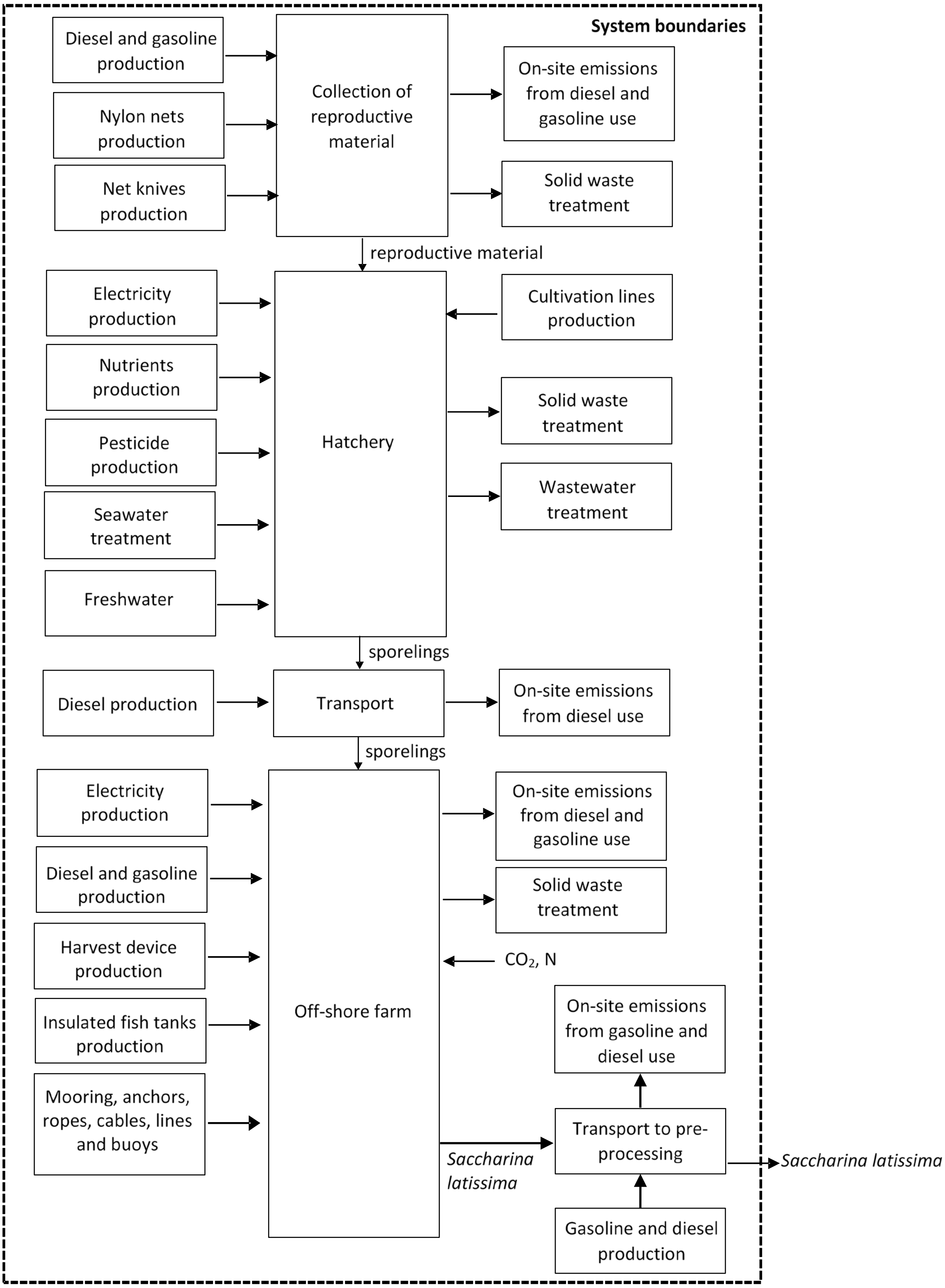

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Figure 3: System boundaries for the current cultivation of Ulva rigida.

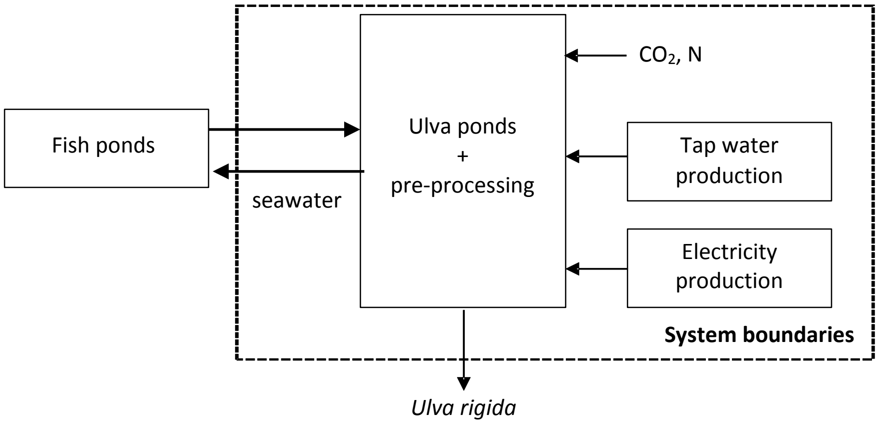

Figure 4: System boundaries for the current processing of Laminaria digitata and future processing of Saccharina latissima.

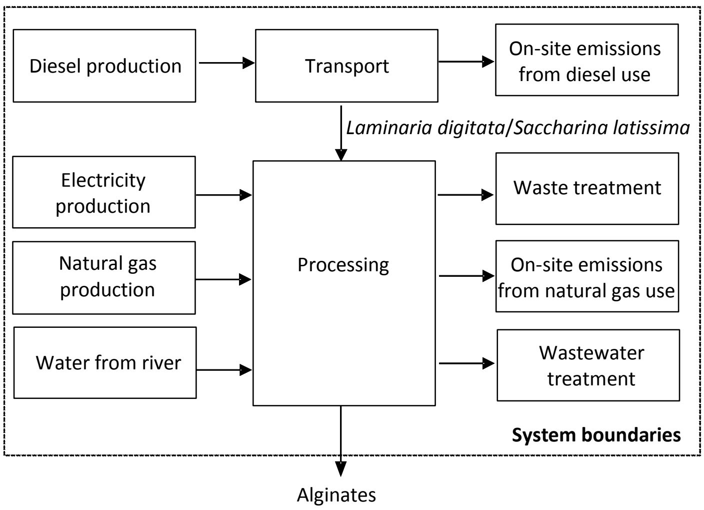

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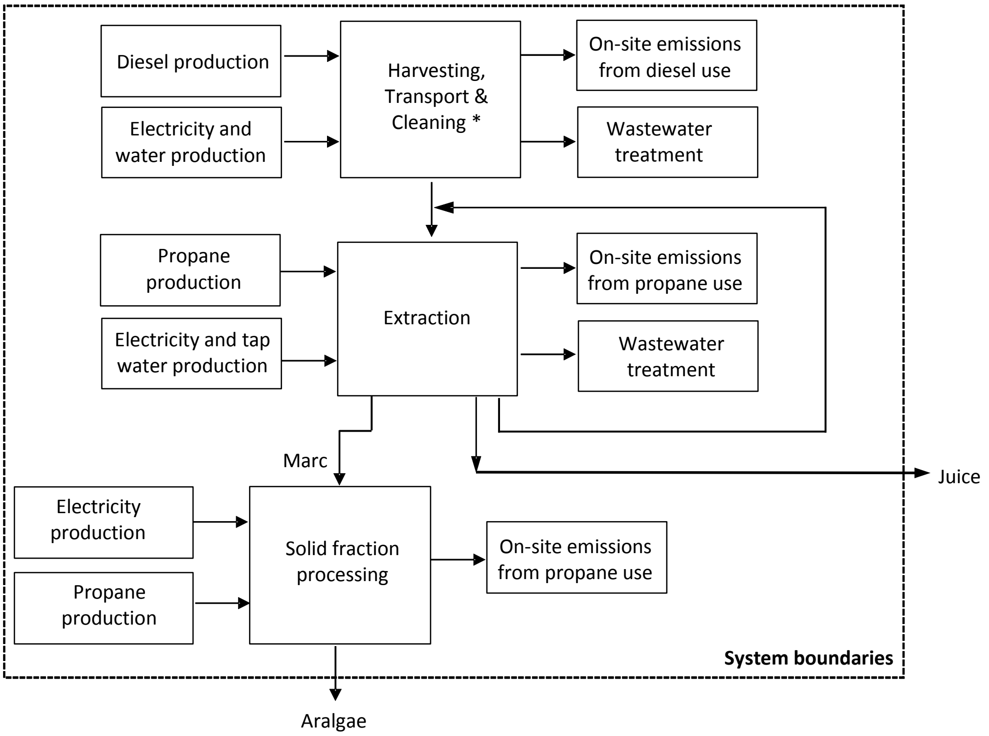
Figure 5: System boundaries for the current processing of Ulva spp and the future processing of Ulva rigida.



* Harvesting, Transport and Cleaning for the current scenario. In the future scenario it includes only Transport and, thus, there is neither electricity and water consumption, nor wastewater treatment.

3.1.1.3 Functional unit

For the *Saccharina latissima* cultivation system, the functional unit is the provision of 1 kg of seaweed (wet basis) without any type of pre-processing. For the *Ulva rigida* cultivation system, the functional unit is the provision of 1 kg of seaweed (wet basis) after washing and centrifugation.

For the processing systems, the functional unit is the processing of 1 kg of seaweed (wet basis) into the production of alginates or Juice and Aralgae.

3.1.2 *Cultivation of Saccharina latissima*

3.1.2.1 Process description

The perennial brown algae *Saccharina latissima* grows throughout the year with a period of maximum growth in the first half of the year followed by a period of reduced growth during summer (Handå et al., 2013). In the fjords of Norway, nutrients (especially ammonia) are well available near salmon farms (Handå et al., 2013). Quite some experience has accumulated in the cultivation of *Saccharina latissima* over the past years, also in southern Atlantic regions (Azevedo et al., 2016; Peteiro & Freire, 2013),

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with potential applications of biofuel (Ghadiryanfar et al., 2016), animal feed and fertilizer (Peteiro & Freire, 2013).

The *Saccharina latissima* for the GENIALG project comes from SES and is grown in off-shore seaweed farms in fjords in middle Norway, near Trondheim. Juvenile sporophytes are obtained from inducing soruses in full-grown blades to release their spores. Natural sorus (spore cluster) formation occurs from late autumn to early winter (Handå et al., 2013) and sporophytes are deployed in fall in the open sea attached to a structure where they grow until harvest in spring.

The current and future cultivation of *Saccharina latissima* can be divided in the following steps:

1. Collection of reproductive material, in which the mother plants are collected manually (with net knives) from nylon nets by means of vessels, and then transported to the hatchery lab by car.

2. Hatchery, in which the release of spores is induced, and the sporelings are allowed to grow in the lab to some millimetres in 1–2 months, in a growing solution containing some fertilizers and pesticide. They attach themselves to thin "seedling ropes" or they are actively attached onto the ropes.

   The hatchery includes a lab and offices, and consumes also electricity, freshwater and seawater that is filtered and treated with sodium hypochlorite solution and ultraviolet (UV) radiation.

3. Transport of sporelings from the hatchery to the port: the sporelings attached to the ropes are transported by car to the port near the off-shore farm.

4. Off-shore farm: two phases can be distinguished, a first one of deployment and growth, followed by harvest. The deployment and growth includes:
   - The seedling ropes are wound around sturdy ropes and deployed in fall in the open sea attached to a structure of buoys, chains and anchoring material.
   - The seaweed grows for 6–7 months until harvest in spring. Deployment and regular checks and maintenance requires significant amounts of transport by boat.
5. Transport of seaweed to the pre-processing is performed with gasoline vessels inside the farm up to dockside delivery, and then with a cooling truck.

The harvest of seaweed can happen both manually and mechanically. Manual harvesting is the most common method globally, and can be used for both natural and cultivated macroalgae. In manual harvesting, devices such as sickle, fork, and net are used to uproot the algae, while mechanized harvesting methods, which can involve mowing, include rotating blades, suction, or dredging cutters (Ghadiryanfar et al., 2016). SES uses some level of mechanization and has adjusted boats and devices to operate, while a significant amount of manual handling of seaweed is required.

3.1.2.2 Primary and secondary data & Inventory modelling

Primary data on the input and output flows associated with each step of current and future *Saccharina latissima* cultivation were provided by SES on an annual basis and then calculated to the functional unit, i.e., 1 kg of seaweed. The uptake of CO_2 and N was estimated based on the carbon and N contents in seaweed biomass, respectively.

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The nutrients added in the hatchery step consist of a modified enriched seawater (ES) medium prepared with seawater and chemicals. The outflow of seawater from the hatchery is assumed to have a quality similar to the seawater used, as nutrients added to the incubation water are all consumed by the sporelings. Wastewater generated from freshwater use at the hatchery is sent to sewage.

The input flows include also materials from the maintenance of the infrastructure of the farm that are replaced. The corresponding material outputs as waste are assumed to have the same end-of-life treatment as municipal solid waste in Norway: recycling (40.7%), incineration (55.8%) and landfilling (3.5%) (OECD, 2020). However, only plastics and steel are recycled, and thus, the UV lamp and nylon cultivation rope, both used in the hatchery, are only incinerated (94.1%) and landfilled (5.9%). Air emissions from the burning of fuels in vessels and road vehicles were estimated based on emission factors from EEA (2016).

Data on the environmental impacts from the production of energy and materials were retrieved from the database Ecoinvent 3.4 (Ecoinvent, 2017). When the exact processes were not available at the database, the most similar ones were selected. No data were available for the production of cartridge filters used for seawater treatment at the hatchery, some of the chemicals used in the preparation of the modified ES medium used at the hatchery, and the production of batteries for electricity generation at the farm. Thus, these processes were not accounted for. However, their contribution is not expected to be relevant for the total impacts.

3.1.3 *Current cultivation of Ulva rigida*

3.1.3.1 Process description

Ulva spp. is suitable for a range of applications since it is rich in sugars and proteins and has a relatively low salt content. Several studies have shown the successful cultivation of *Ulva* spp. under IMTA conditions (Korzen et al., 2016; Marinho et al., 2013; Mata et al., 2006).

The *Ulva* biomass for the GENIALG project comes from ALGAplus and is grown in a land-based IMTA system. Nutrient rich water from the semi-intensive sea bream and sea bass farm provides the nutrients required. The water in the seaweed tanks is bottom-aerated with electricity powered turbines to facilitate nutrient assimilation and access to light. High biomass yields per surface area can be achieved without adding CO_2 or fertilizers. The cultivation of *Ulva rigida* is carried out according to the following steps:

1. Starting stock: clonal propagation is practiced so that no sexual reproduction is required, precluding a hatchery cycle in the propagation. Cultivation is started with a “starting stock” of a population of genetically related individuals from natural occurrences within the property or from the previous cultivation cycle. Small portion of individual blades are maintained in controlled culture rooms in order to preserve the strains of *Ulva rigida* in cultivation. The support lab including the offices that support the R&D and hatchery operations requires electricity (artificial light, equipment functioning) and water for lab and office operations.
2. Growth phase: growth occurs in IMTA conditions from moulded plastic tanks of 1000 L up to concrete tanks of 20.000 L scale, with a production capacity of up to 40 t (fresh weight) of Ulva per year. The free-floating seaweed are kept in circulation by bottom aeration.

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3. Harvest is done by draining the tanks and harvesting the seaweed with net-tools. The seaweed is collected and transported in perforated plastic boxes. This transport was excluded in the present study.
4. Pre-processing: the washing process uses seawater filtered down to 5 micron, UV sterilized and ozone treatment. The seaweed passes through a water bath with strong agitation, followed by spraying it in a conveyor belt system. The wet seaweed is dewatered in a centrifuge. This pre-processing requires electricity.

3.1.3.2 Primary and secondary data & Inventory modelling

Primary inputs data associated with *Ulva rigida* cultivation were provided by ALGAplus on an annual basis for the year 2019 and then calculated to the functional unit, i.e., 1 kg of seaweed. The uptake of CO_2 and N was estimated based on the carbon and N contents in seaweed biomass, respectively. Ecoinvent 3.4 was the source of the data on the environmental impacts of electricity and tap water production.

3.1.4 *Current processing of Laminaria digitata and future processing of Saccharina latissima*

3.1.4.1 Process description

During most time of the year, currently Algaia is processing directly fresh brown seaweed from the coasts of Brittany in France. In the future scenario, Algaia is supposed to receive seaweed from Seaweed Solutions. The current and future processing at Algaia takes the following basic steps:

1. Transport: wild *Laminaria digitata* is transported by truck from the beach to the processing factory, whereas *Saccharina latissima* from Seaweed Solutions is assumed to be transported by sea (2,155 km) and by truck (25 km).
2. Lixiviation: the fresh seaweed is cut and directly sent to a first tank for acid lixiviation.
3. Maceration: the main solid seaweed material is then sent for extraction bioreactors where the maceration phase takes place in alkaline conditions.
4. Precipitation: the alginate concentrate precipitates in acid conditions to reform the alginic acid fibers.
5. After this precipitation, fibers are pressed to remove water and particles, washed again, pressed, neutralized with a salt and then sent for drying and milling. The end products are alginates in powders of different particle size.

3.1.4.2 Primary and secondary data & Inventory modelling

Primary data on the input and output flows associated with each step of current *Laminaria digitata* processing were provided by Algaia on an annual basis and then calculated to the functional unit, i.e., 1 kg of seaweed (wet basis). All data, except those related with the seaweed transport, are equal when expressed by functional unit in the two scenarios. Air emissions from the burning of natural gas were estimated based on emission factors from EEA (2016). Ecoinvent 3.4 was the source of the data on the environmental impacts of transport, and electricity and natural gas production.

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3.1.5 *Current processing of Ulva spp. and future processing of Ulva rigida*

3.1.5.1 Process description

Olmix produces food additives for humans, feed additives for animals and stimulation products for plants, currently from wild harvested green and red seaweed from the coasts of Brittany in France. The seaweed contains macromolecules and trace elements that are important for OImix' products.

The production at Olmix takes the following basic steps:

1. Harvesting, transport and cleaning: currently the *Ulva* spp. is harvested from the beach in a special truck by a contractor located in Plouénan. The seaweed is washed with well water from a dedicated treatment plant. Sand and crustaceans are removed, and the seawater adjacent to the seaweed is removed. The washed seaweed is then delivery to Bréhan by truck for processing. For the future processing of *Ulva rigida*, only transport by truck from ALGAplus to Olmix facilities are included in this first step.
2. Extraction: the seaweed is ground and then submitted to an extraction process resulting in a solid and a liquid phase. The liquid phase, excluded from the system boundaries, is sent off-site for formulation into end products.
3. Solid fraction processing: the dry 'cake' (solid fraction) from the extraction is used as a protein-rich macroingredient. It is mixed with montmorillonite clay and dried on-site with propane gas consumption and packed. The evaporated water is removed as steam.

The plant site also contains a laboratory where the same process steps can be accomplished at a small scale. This way the extraction process can be closely monitored, modified and tested, giving technicians the flexibility to adapt the treatment the seasonally and spatially variable chemical composition of the seaweed. Each step also consumes electricity for mechanised operations.

3.1.5.2 Primary and secondary data & Inventory modelling

Quantitative data on propane gas, electricity, water and transport use were provided by Olmix. For the extraction and solid fraction processing steps, the data expressed by functional unit are equal in the two scenarios. Air emissions from the burning of propane were estimated based on emission factors from EEA (2016). Ecoinvent 3.4 was the source of the data on the environmental impacts of transport, and water, electricity and propane production.

3.2 Results and discussion

3.2.1 *Cultivation of Saccharina latissima*

Figure 6 compares the total impact assessment results of the current and future cultivation of *Saccharina latissima* and presents the contributions of each step. The future scenario is environmentally better than the current one in all impact categories. When only impacts are considered (i.e., excluding uptake of CO_2 and N), the decrease in the impacts ranges from 44% to 63%, depending on the category. These decreases result from environmental improvements in all the steps (i.e. the impact is up to 37-60% lower in hatchery, 48-76% in farm, and 20-22% in transport to pre-

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processing), except in the transport between hatchery and port, which remains the same. The uptake of CO_2 and N contribute positively (negative contributions) for global warming and marine eutrophication, respectively, in both scenarios. In fact, resulting negative emissions from carbon capture and bioremediation are considerable compared to the positive emissions. In the case of global warming, uptake is 1.1 and 2.3 times higher than impacts in the current and future scenarios, respectively. In marine eutrophication, the offset is even more remarkable with uptake exceeding impacts 669 and 1,128 times in the current and future scenarios, respectively. It should be noted that these uptakes are only temporary as CO_2 and N will be released from the seaweed later in the value chain. However, if the N is released to reservoirs other than seawater, freshwater and agricultural soil, it will not contribute to marine eutrophication but rather to other impact categories (Huijbregts et al., 2017). For example, if N is emitted to air as nitrogen oxides (NO_x) it would contribute to ozone formation and terrestrial acidification, or if N is emitted to air as ammonia (NH_3) it would contribute to terrestrial acidification.

For both scenarios, the step of off-shore farm has the largest contribution to the impacts in all impact categories, except marine eutrophication, representing 70 to almost 100% and 54% to almost 100% of the total impacts, for current and future scenario, respectively, depending on the impact category. The hatchery has the highest contribution in marine eutrophication (83 and 89%, in current and future scenario, respectively), and ranges from 29-42% in freshwater eutrophication, 7-12% in global warming and fossil resources scarcity, and less than 2% in the remaining impact categories for both scenarios. The share of the remaining steps is relatively small. The impacts of collection of reproductive material is lower than 4% in all categories. The impacts of the transportation of the sporelings from the hatchery to the port contribute less than 3% for the impacts for both scenarios, except for global warming and fossil resource scarcity for which it represents 5 and 10% of the total impacts for current and future scenario, respectively. Regardless of the scenario, the impacts of the transportation to the pre-processing contribute to 7-13% of the impacts, except in freshwater and marine eutrophication and mineral resource scarcity for which it represents less than 2% of the total impacts.

The impacts of the step of collection of reproductive material are the same for both scenarios. Figure 7 shows that the diesel use in vessels dominates the impacts of this step in four of the seven impact categories (global warming, ozone formation, terrestrial acidification and fossil resources scarcity), ranging from 56 to 90%. Nylon nets cause the largest impact in freshwater and marine eutrophication (67 and 93%, respectively), and steel of net knives up to 90% in mineral resource scarcity. The solid waste treatment leads to a decrease of the impacts (negative contributions) in five impact categories, mainly in mineral resource scarcity (almost 6%), because material recycling avoids the consumption of virgin material.

Figure 8 shows that the impacts of the hatchery are dominated by electricity use in five of the seven impact categories, ranging from 85% to 99% of the total impacts for both scenarios. Wastewater treatment (in particular, the discharge of treated wastewater) is the main contributor to freshwater (55 and 66%, for current and future scenario, respectively) and marine eutrophication (97-98% for both scenarios).

Figure 9 shows that diesel use in vessels dominates the impacts of the off-shore farm in four impact categories (global warming, ozone formation, terrestrial acidification and fossil resource scarcity), representing 41-88% for current scenario and 53-93% for future scenario. The anchor chains are the main contributor to mineral resource scarcity (97% and 94% for current and future scenario, respectively) and freshwater eutrophication (82% and 67% for current and future scenario,

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respectively), and ammonium ion emissions from solid waste treatment contributes up to 56% (current scenario) and 40% (future scenario) in marine eutrophication. Solid waste treatment also leads to a decrease of the impacts (negative contributions) in six impact categories, mainly in mineral resource scarcity (14-16%) for both scenarios.

Regardless of the scenario, Figure 10 shows that the impacts of transport to pre-processing are dominated by gasoline use (72-98%) in the vessels inside the farm. Transport by refrigerated truck has a contribution up to 20-28% in global warming, freshwater and marine eutrophication, and fossil resource scarcity.

Figure 6: Contributions of the steps of the current (C) and future (F) cultivation of Saccharina latissima, considering 100% or -100% for the scenario with the highest or lowest burden, respectively, in each impact category.

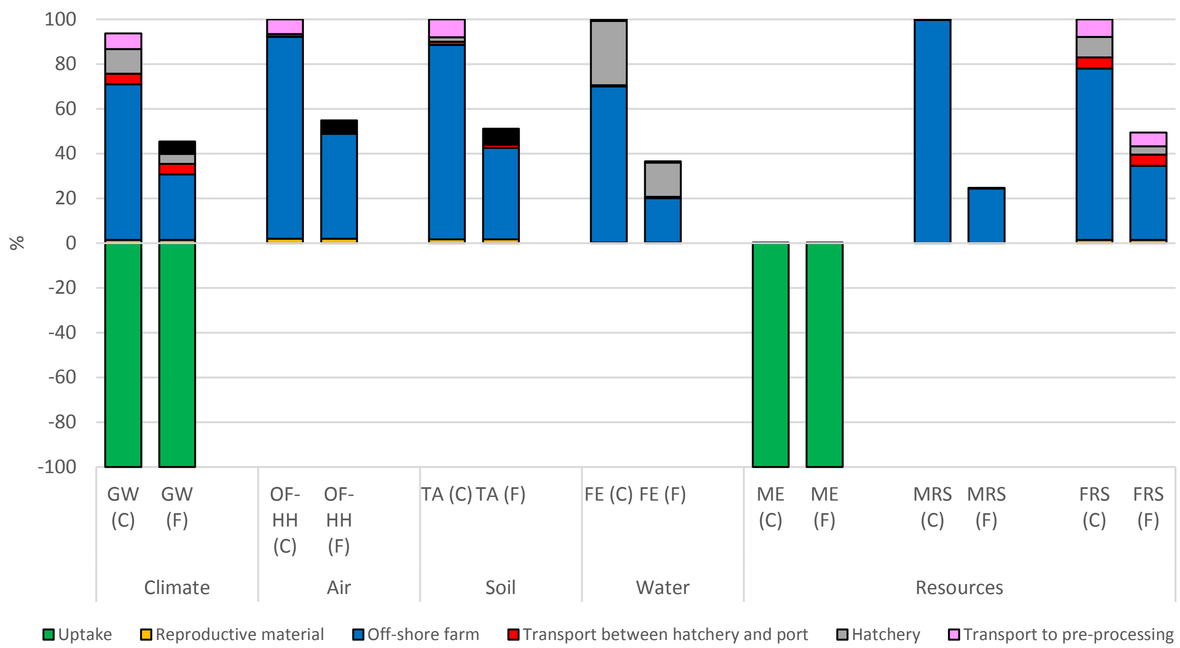

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Figure 7: Contributions of the processes in the step of collection of reproductive material of Saccharina latissima for both current and future scenarios.

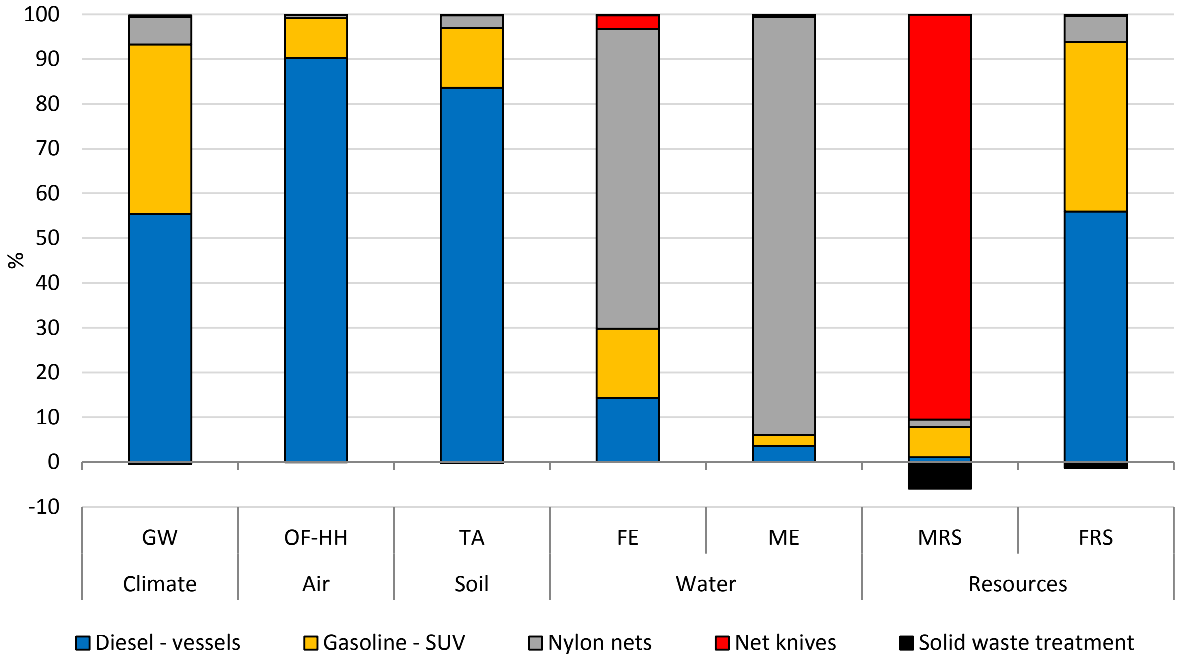

Figure 8: Contributions of the processes in the current (C) and future (F) step of hatchery of Saccharina latissima, considering 100% for the scenario with the highest burden in each impact category.

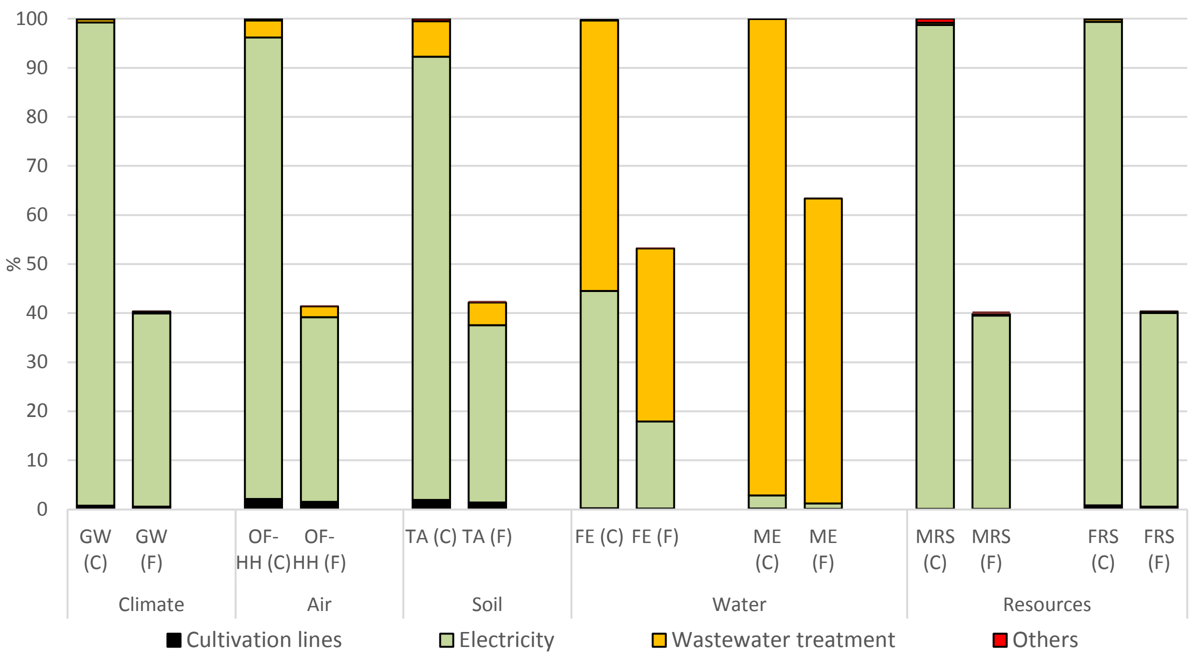

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Figure 9: Contributions of the processes in the current (C) and future (F) step of off-shore farm of Saccharina latissima, considering 100% for the scenario with the highest burden in each impact category.

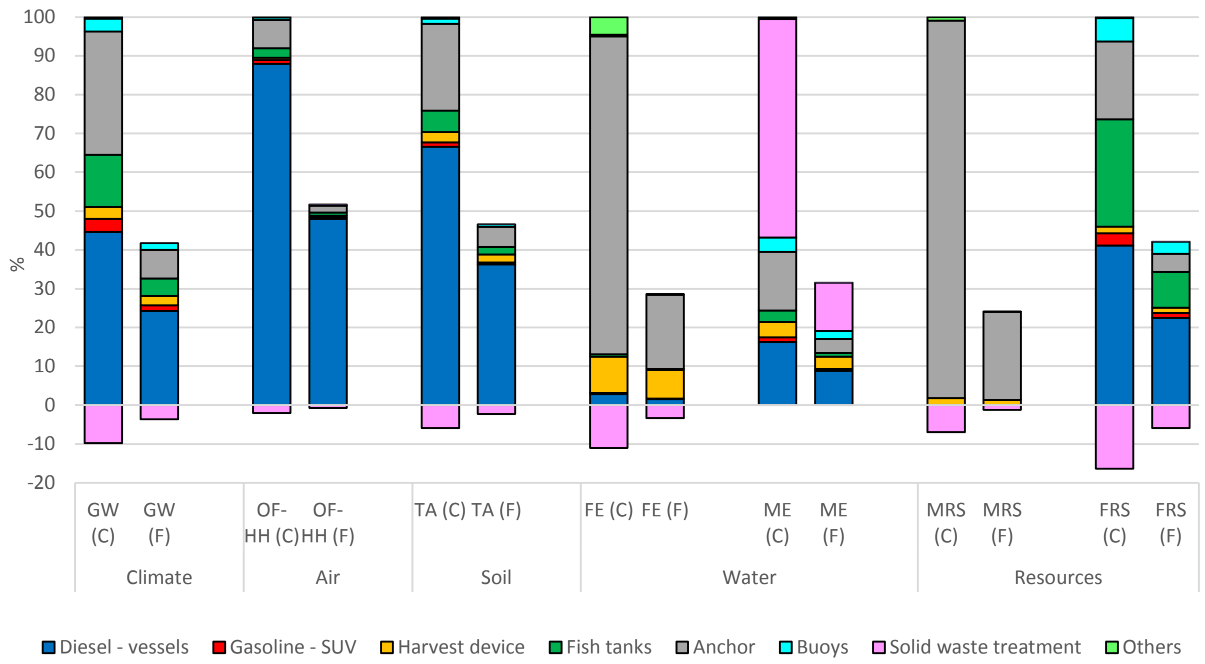

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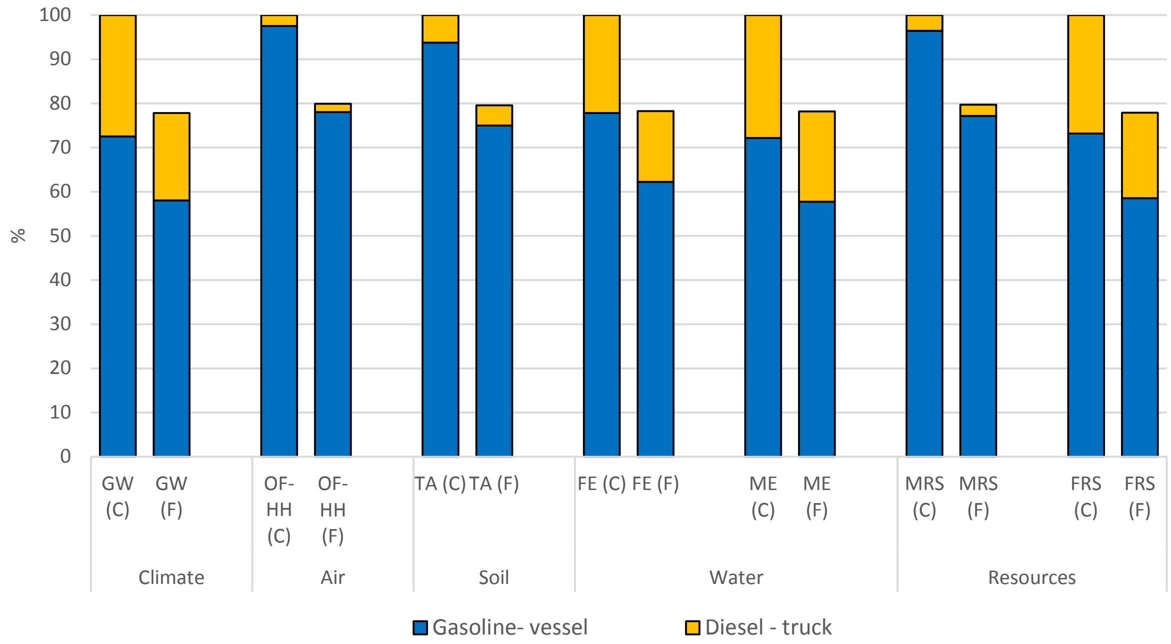
Figure 10: Contributions of the processes in the current (C) and future (F) step of transport to pre-processing of Saccharina latissima, considering 100% for the scenario with the highest burden in each impact category.



3.2.2 *Current cultivation of Ulva rigida*

The contributions obtained for the inputs and outputs of *Ulva rigida* cultivation to the total impacts are presented in Figure 11. The use of electricity contributes to almost 100% of the impacts in all the categories, except in marine eutrophication, where water emissions have a contribution of almost 100%. In this category, N uptake (bioremediation) by seaweed contributes to mitigate (temporarily) the impacts, being 2.7 times the N equivalent emissions to the sea, resulting in a net uptake. In global warming, uptake also mitigates (temporarily) the impacts up to 10% of total CO_2 equivalent emissions.

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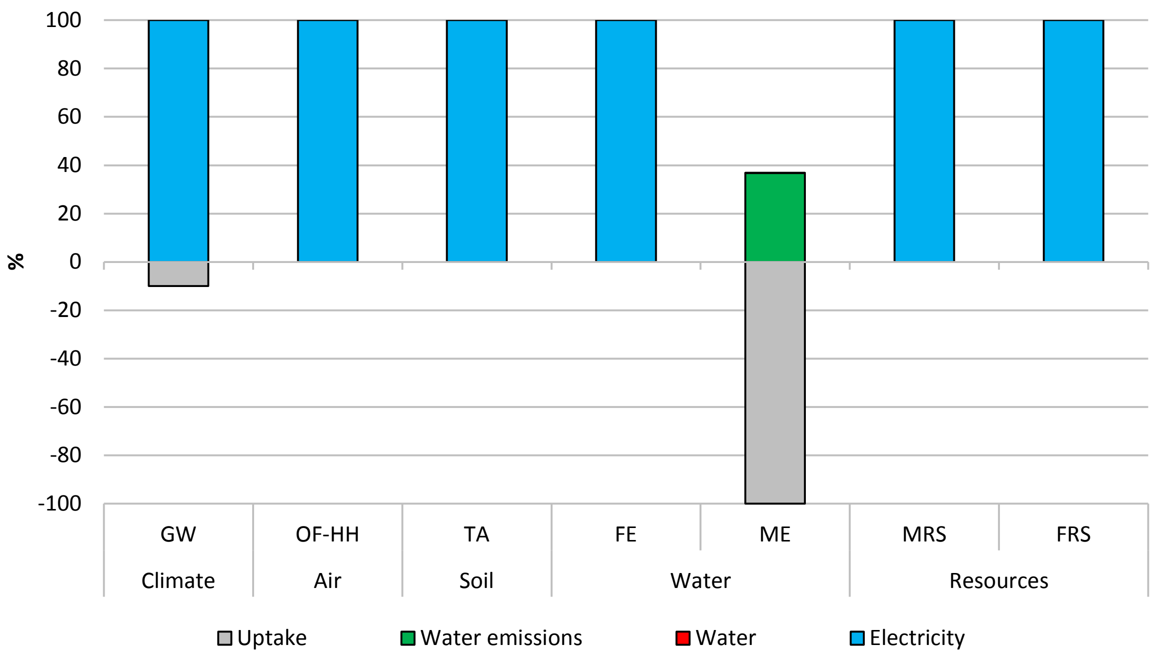
Figure 11: Contributions of inputs/outputs to the environmental impacts of Ulva rigida cultivation, considering 100% or -100% for the scenario with the highest or lowest burden, respectively, in each impact category.



3.2.3 *Current processing of Laminaria digitata and future processing of Saccharina latissima*

Figure 12 shows the impact assessment results for the current processing of *Laminaria digitata* and future processing of *Saccharina latissima.* Future processing is environmentally worse than the current one in all impact burdens as a result of the highest transport distance. The difference is particularly high for ozone formation and acidification, for which the current scenario represents only 8% and 21% of the impacts of the future scenario. However, in the categories of freshwater and marine eutrophication and mineral resource scarcity, the differences are negligible (less than 0.5%).

For the current scenario, the step of processing has the largest contribution to all impact categories, representing almost 100% of the total impacts (Figure 12). For the future scenario, the processing has the highest contribution in the five of the seven categories (from 76 to almost 100%), and the transport is the main contributor in ozone formation and acidification (ranging from 79 to 92%) due to the high emissions of NO_x and sulphur dioxide (SO_2) during transport between Seaweed Solutions (Norway) and Algaia (France).

Figure 13 shows that the impacts of the processing step (similar for both scenarios) are dominated by natural gas (pre-combustion and combustion) in four of the seven impact categories (global warming, ozone formation, terrestrial acidification and fossil resource scarcity), ranging from 67 to 94%. Water emissions causes the highest burdens in freshwater and marine eutrophication (89 and 80%, respectively) due to phosphorus (P) and N emissions. Electricity contributes to almost 100% in mineral resource scarcity, because the French electricity mix is mainly dominated by the nuclear energy (uranium consumption).

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Figure 12: Contributions of process steps to the environmental impacts of Algaia products, considering 100% for the scenario with the highest burden in each impact category. C: current processing of Laminaria digitata. F: future processing of Saccharina latissima

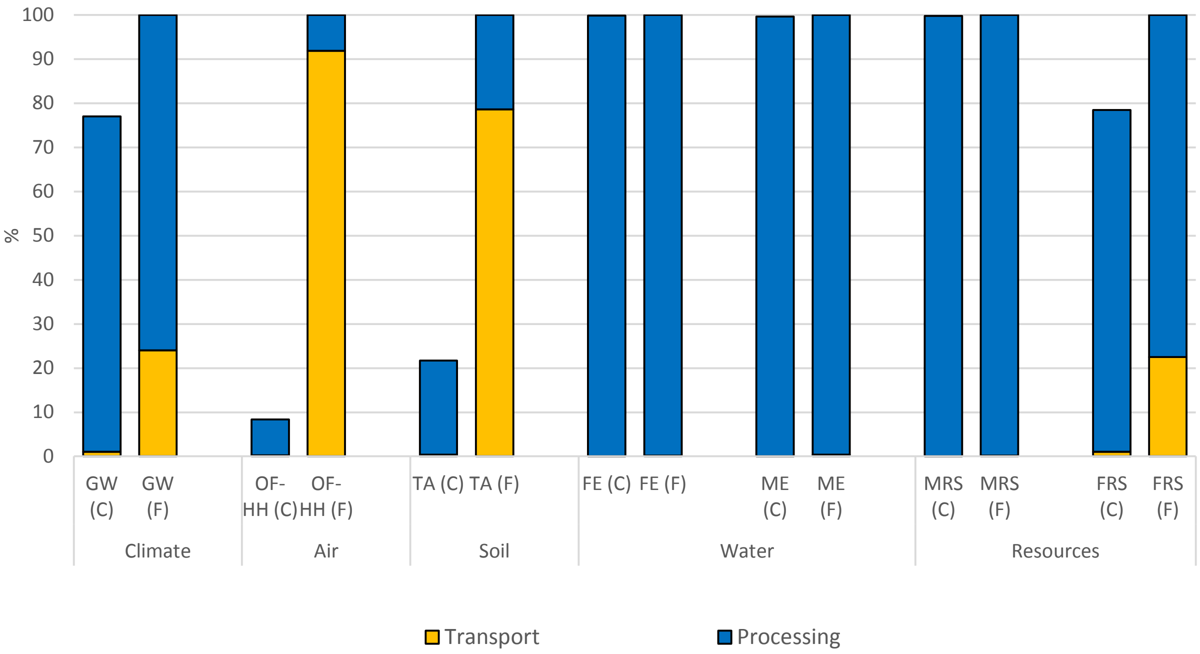
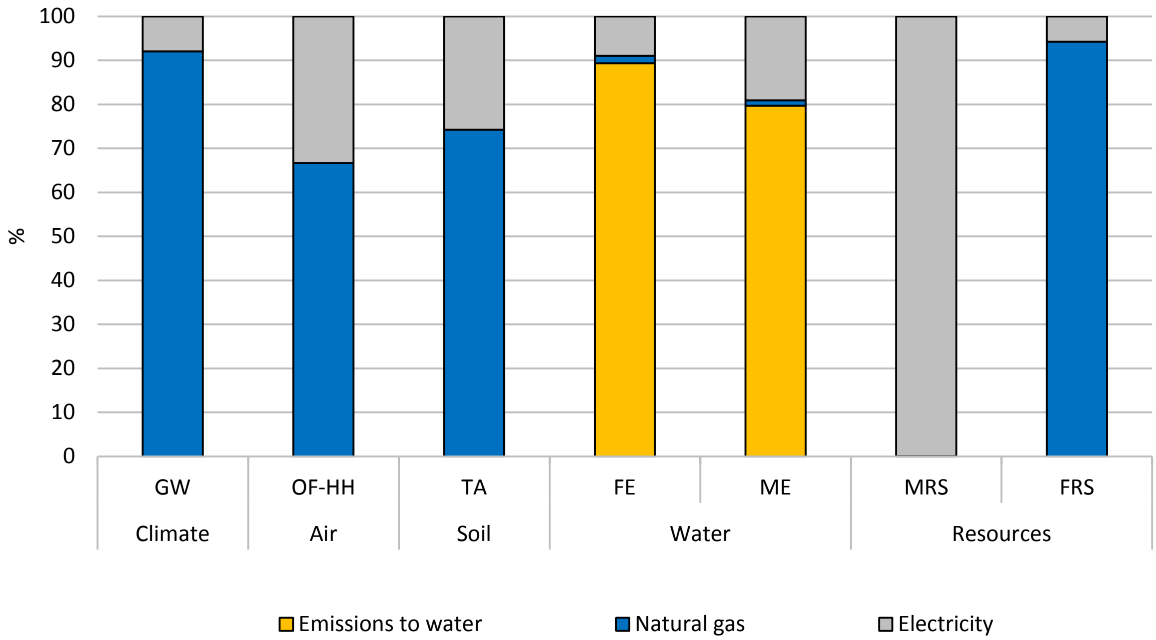


Figure 13: Contributions of the processes in the step of processing of Algaia products.



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3.2.4 *Current processing of Ulva spp. and future processing of Ulva rigida*

Figure 14 shows the contributions of each step for the total impacts associated with the current processing of *Ulva* ssp. and future processing of *Ulva rigida* from ALGAplus by Olmix. In Figure 15, the contributions from the inputs and outputs are shown for both scenarios. The future scenario is environmentally worse than the current one in four of the seven impact categories (increase of 9 to 17%) as a result of the highest transport distance from ALGAplus (Portugal) to Olmix (France) facilities, but it is environmentally better (decrease of 21 to 74%) in water-related burdens and mineral resource scarcity because Ulva from ALGAplus does not require neither harvesting nor cleaning at Olmix facilities.

The step of solid fraction processing has the largest impact in four of the seven categories, i.e. global warming, ozone formation, terrestrial acidification, and fossil resource scarcity (47-56%), for both scenarios, mainly due to the natural gas used in this step. The extraction step is the main contributor in mineral resource scarcity (51-66%) for both scenarios, due to electricity consumption (French electricity mix, predominantly based on nuclear energy). The harvesting, transport and cleaning has the largest burdens in both water-related eutrophication categories for the current scenario (53-76%) due to P and N emissions discharged to the environment after wastewater treatment. In the future scenario, where the first step only includes transport and, as a result, there is neither electricity and water consumption, nor wastewater treatment associated with harvesting and cleaning, the extraction step is the main contributor to both eutrophication categories (68-87%) also due to P and N emissions.

Figure 14: Contributions of process steps to the environmental impacts of Olmix products, considering 100% for the scenario with the highest burden in each impact category. C: current processing of Ulva spp. F: future processing of Ulva rigida.

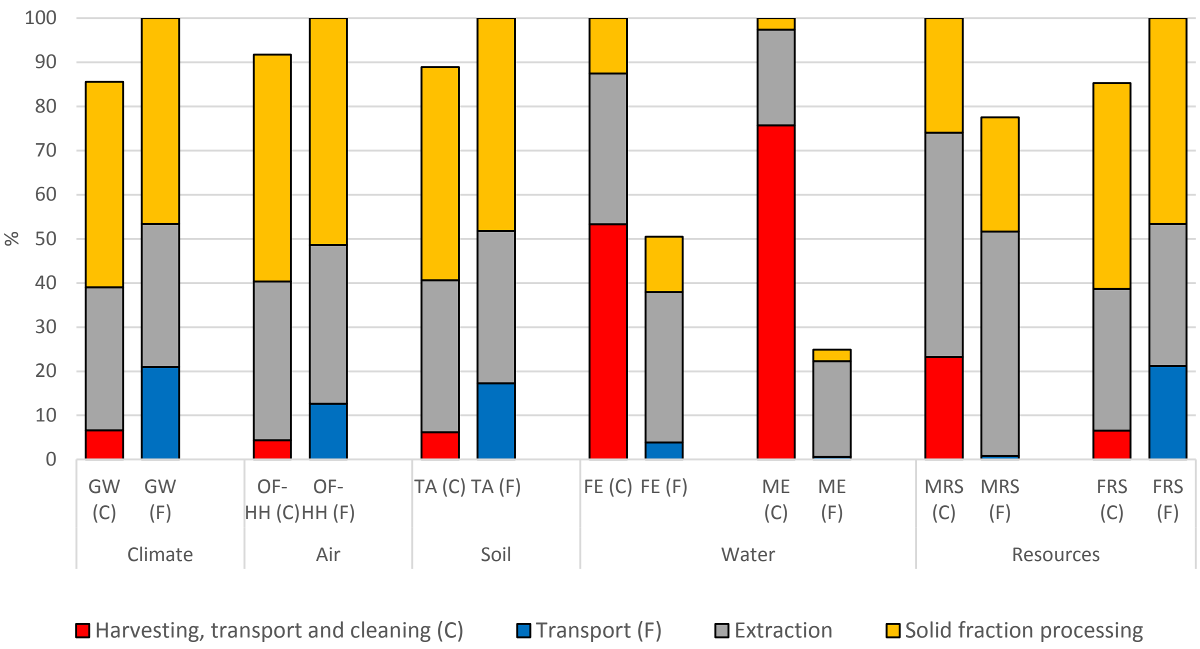

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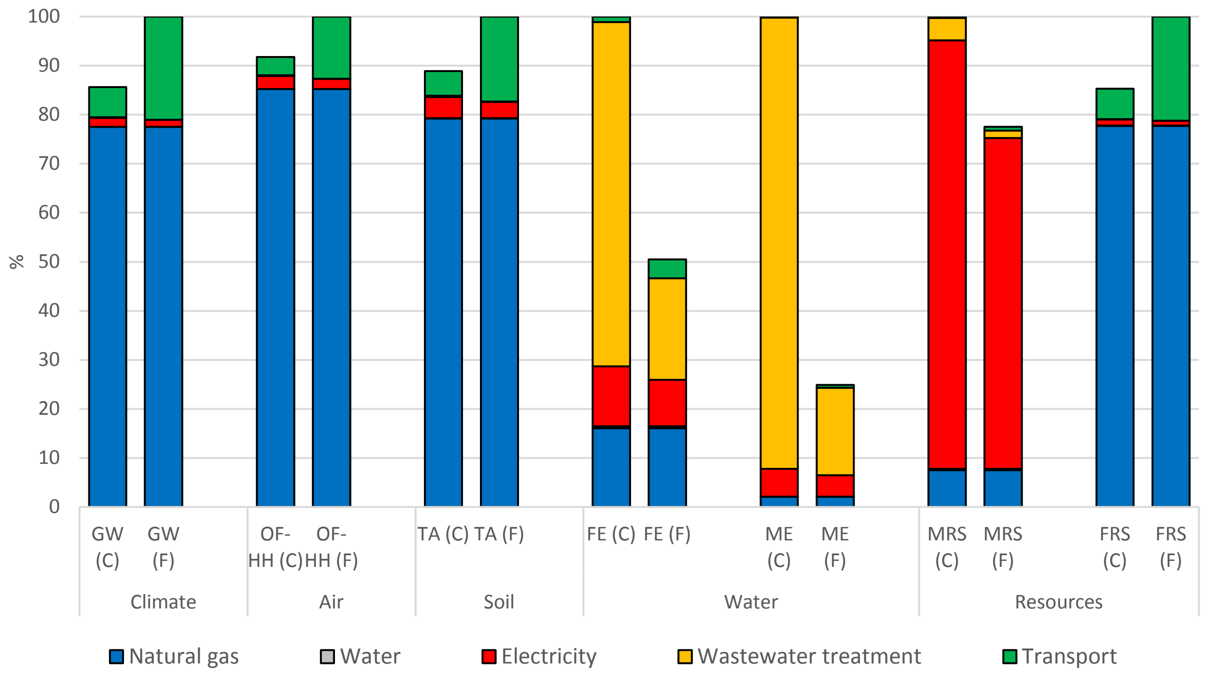
Figure 15: Contributions of inputs/outputs to the environmental impacts of Olmix products, considering 100% for the scenario with the highest burden in each impact category. C: current processing of Ulva spp. F: future processing of Ulva rigida.



3.3 Conclusion

Part I of this report presents the final LCA of the current and future cultivation of *Saccharina latissima* by Seaweed Solutions, current cultivation of *Ulva rigida* by ALGAplus, current processing of wild *Laminaria digitata* and future processing of *Saccharina latissima* by Algaia, and current processing of wild *Ulva* spp. and future processing of *Ulva rigida* by Olmix. The results obtained from this assessment highlight the main environmental hotspots and allow to anticipate how the impacts will evolve in the future.

The main environmental hotspots in current and future *Saccharina latissima* cultivation derive mainly from the use of diesel and steel anchor chains in the farm and from N emissions to water in the hatchery. Impacts are expected to decrease by 44-63% in the future scenario, due to environmental improvements in all the steps other than transport between hatchery and port, which remains the same. The improvements are particularly relevant in the off-shore farm mainly as result of less diesel use in vessels for several activities such as harvest, deployment, maintenance and inspections and by less amount of steel used in anchor chains to produce 1 kg of seaweed. The impacts from *Ulva rigida* cultivation are almost exclusively associated with the use of electricity. Both cultivation systems have mitigation of impacts in global warming and marine eutrophication as a result of CO_2 and N uptake by seaweed. However, it should be emphasized that these benefits are temporary and the CO_2 and N will return as emissions to the environment in the subsequent stages of the life cycle (processing, use and end-of-life), but N will only contribute to marine eutrophication impacts if it is emitted to seawater, freshwater and agricultural soil, otherwise it will contribute to other impact categories.

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In the current processing of wild *Laminaria digitata* at Algaia, the main contributions are from the use of natural gas and electricity during processing, as well as from N and P emissions to water in eutrophication-related categories. Future processing of *Saccharina latissima* at Algaia facilities (assuming that the environmental burdens of the processing step per 1 kg of seaweed remain the same as currently) will result in higher impacts due to the larger transport distance. The impact increase is more relevant in ozone formation and acidification (12 and 4.6 times, respectively), for which the transport is the main hotspot, and almost negligible in eutrophication and mineral resource scarcity.

In the current processing of wild *Ulva* spp. at Olmix facilities, the largest contributions come from the consumption of gas and electricity during solid fraction processing and extraction steps, respectively, and also from the emission of N and P to water. The environmental impacts of the future processing of *Ulva rigida* at Olmix facilities (considering that the environmental burdens of the processing step per 1 kg of seaweed remain the same as currently) are worse than in the current processing of *Ulva spp.* in four of the seven impact categories (increase of 9-17%) due to the higher transport distance. However, the impacts in freshwater and marine eutrophication and in mineral resource scarcity will decrease by 49%, 74% and 21%, respectively, because seaweed cleaning is no longer performed at Olmix facilities.

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4 PART II: LCA OF NEW SEAWEED APPLICATIONS

4.1 Methodology

4.1.1 Goal and system boundaries

The goal of part II of this report is to map the environmental impact of individual or combined processing steps of new seaweed applications and identify hotspots within the steps. This environmental impact information can inform the further scale-up of individual steps and the development of biorefinery designs. For the enzymatic treatments the effect of moving to the next scale level and two more upscaling options will be studied.

In general, a lot of information on process and product characteristics has been collected in GENIALG but a full biorefinery design with a process parameterization is not designed. Most fractions produced in the project could have different functions or functions are unknown, and hence different configurations are being considered. Industrial partners could implement a novel processing step in their processes to provide a new fraction to a third party who uses it in its formulations. Moreover, process steps at the lab scale need to be scaled up, both independently and in interaction with the overall biorefinery.

The processing steps considered are the enzymatic treatment and the extraction of carotenoids:

- Enzymatic treatment is a two-step process with wet or dried seaweed as an input and it is always followed by a phase separation. These steps are considered for the enzymatic treatment, including seaweed drying. The resulting fractions contain water. The removal is not required for local further processing and the amount to be removed for transport off-site is not known, so drying is out of scope.
- Extraction of carotenoids requires drying before the supercritical carbon dioxide extraction, while downstream processing is flexible and undecided. Thus the drying and the extraction are included in the analysis.

In all systems, the production of energy, fuels, water and materials consumed in all the processes was also considered. The production of capital goods (buildings, machinery and equipment) was excluded since estimations would be highly uncertain and their contributions minimal. System boundaries are set as the assessment on the processing only, excluding seaweed cultivation and downstream processing. The system boundaries are illustrated in the method sections specific to these two analyses.

4.1.2 Enzymatic treatment

4.1.2.1 Products and functional unit

In GENIALG, a double enzymatic treatment was piloted on a large lab scale (85 L) with different enzyme combinations for both seaweed species. This process produces four interesting fractions with different applications of interest to the GENIALG consortium partners (Figure 16):

1. After the first enzymatic treatment, a fraction of the seaweed dissolves into the process water. The liquid could be concentrated for formulation in a liquid product.

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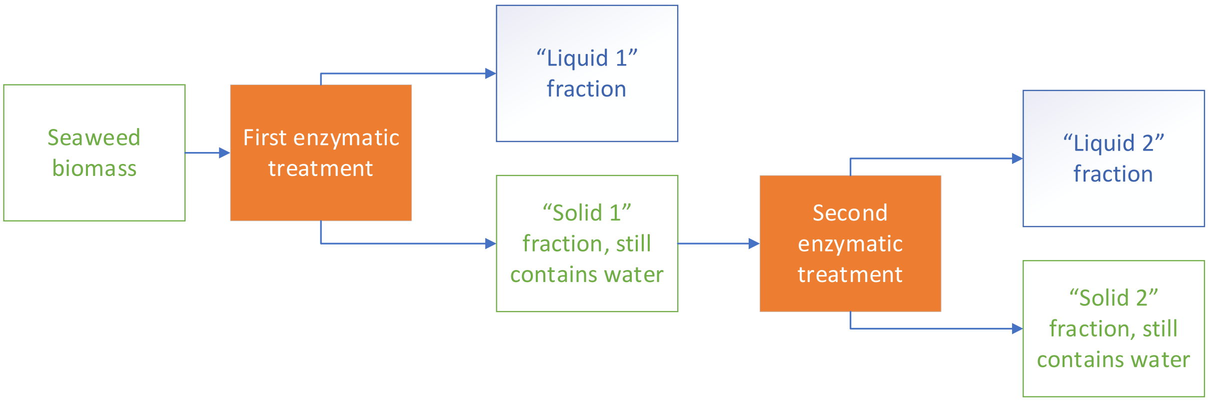
2. Another fraction can be separated from this liquid and can be called “solid”. It does contain water unless it is dried. The solid/liquid separation is done through filtration with a mesh bag on labscale (BDC, 2021) or centrifugation on larger scale (Olmix, 2021).
3. This solid is treated a second time and yields a liquid.
4. As well as a solid.

Figure 16: Overview of the two steps in the double enzymatic treatment and the resulting fractions.



The liquid from the second enzymatic treatment will be tested for cosmetic applications. This liquid will be central to the presentation of the result, but the impact of the other fractions has been modelled and will receive some attention. With the functions of the fractions unknown but of interest, comparing the fractions with each other and between the scenarios is useful, which should be done in a fair manner. The activity of a fraction derives from the compounds within the fraction and not from the water content, so the dry matter mass in each fraction was used as a functional unit. The treatment of a quantity of seaweed was a second functional unit, as justified by the goal of understanding the processing. Impacts will be expressed respectively in terms of:

- 1 kg dry matter of each fraction,
- treatment of 1 ton of seaweed, with the total mass in kg of dry matter produced of different fractions reported.

4.1.2.2 Dealing with alternative process flows and multifunctionality

Each enzyme treatment yields two fractions. One intermediate fraction (Solid 1) and three final fractions are created (Liquids 1, 2 and Solid 2), and the impact of individual fractions is required for comparisons. The overall environmental impact of the treatment process is also of interest, and attribution of the impacts is not required for this, but it will be illustrative. It should be determined how the environmental impact of the system studied ends up at the four fractions.

Out of three options to approach the issue of multifunctionality, process subdivision (first option) and substitution/system expansion (second option) should be considered before allocation. This is the third option according to the ISO standard on LCA (ISO, 2006). However, processes in this study are fully subdivided. The second option means adjusting the system boundaries of the LCA model to account for functions of the coproduct with less focus. The functions of the products should be known and to a lesser extent, the product of focus should be known. Since the relative importance and the function

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of the four fractions are unknown, the second option is dropped. Thus, the environmental impact of each treatment should be allocated between the two fractions it creates.

The allocation should follow the function of both the coproducts, which are unknown in this case. The dry matter content predicts the function to some extent and was chosen as a basis for allocation. The allocation and the reference flow are based on the same quantity which implies that 1 kg of dry matter of liquid and 1 kg of dry matter of solid from the same enzyme treatment will have the same environmental impact.

4.1.2.3 Scenarios

In discussions with BDC (2021) the basic steps of the enzyme treatment were identified, the modelling approach and results were discussed. The lab results on yields of liquid and solid fractions were shared. Since the function of the fractions is not known and may vary for different applications, the choice of enzymes could not be defined beforehand and the averaged amount of enzymes was used as a variable in the model, instead of the type and specific use amount.

The lab results for both seaweed species was a starting point (Scenario (S) 1 and 2). In order to inform further scale up, three scenarios on a 10 times larger scale were modelled with different options (S4-6). Because these three scenarios are based on a theoretical approach from Piccino et al. (2016), a scenario that function as theoretical reference was drawn up (S3). The following scenarios were defined, with their dry matter yields reported in Table 1:

1. Double enzyme treatment of *Saccharina latissima* on lab scale according to the lab data.
2. Double enzyme treatment of *Ulva rigida* on lab scale according to the lab data.
3. Double enzyme treatment of an average seaweed on lab scale with calculated energy consumptions, with averaged yields from the lab data.
4. Double enzyme treatment of an average seaweed on a 10 times larger scale with calculated energy consumptions.
5. Double enzyme treatment of an average seaweed on a 10 times larger scale with calculated energy consumptions, but with a shorter incubation time with an enzyme-deactivation period afterwards at elevated temperature.
6. Simultaneous treatment of an average seaweed with two enzymes on a 10 times larger scale with calculated energy consumptions.

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Table 1. Absolute yields in dry matter (DM) amount from double enzyme treatment scenarios (Solid 1 is excluded from the total because it is consumed to obtain liquid 2 and solid 2).

Treating 1 ton of seaweed yields...		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
		Lab Scale, *Saccharina latissima*	Lab Scale, *Ulva rigida*	Lab Scale, Averaged	Upscaled	Upscaled - Short	Upscaled - Simult.
Liquids 1	Kg	70.4	66.8	66.4	66.4	66.4	0.0
Solids 1	kg	66.4	65.3	63.8	63.8	63.8	0.0
Liquids 2	kg	19.5	20.5	19.4	19.4	19.4	85.8
Solids 2	kg	46.9	44.8	44.4	44.4	44.4	44.4
Total DM exc. Sol. 1	kg	137	132	130	130	130	130

4.1.2.4 Process description

The process consists of the following steps, as illustrated in Figure 17:

1. Wet seaweed is dried for S1-3 or ground for S4-6.
2. Water is added to arrive at the reaction volume. Acid and enzyme are added. Two types of enzymes are added for S6.
3. Heating is started and a set temperature is maintained for the incubation time, under stirring.
4. After incubation, a deactivation period is included for S5.
5. The solids are separated from the liquids through manual filtration with a mesh bag for S1-3 and through centrifugation for S4-6.
6. The solids are transferred to a new reaction vessel and steps 2-5 are repeated for S1-5.

All solids and liquids contain substantial amounts of water and can be dried with different methods. This is not included in the environmental impact results, but the heat requirements were calculated, and reported in Table 2. Data for ultrafiltration was derived from Sharaai et al. (2009), Avula et al. (2009), Wernet et al. (2016), Pavez et al. (2015) and Glucina et al. (1998). Avula et al. (2009) focused on microalgae while the other sources focused on water purification. Data for spray drying was derived from Kyriakopolou et al. (2015) and Perez-Lopez et al. (2014). Oven drying was calculated based on the heat capacity of water and the oven drying efficiency of Piccino et al. (2016). These energy demands will be addressed in the discussion.

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Table 2. Energy requirements for drying selected fractions from double enzyme treatment scenarios, expressed per ~4.17 kg DM of seaweed treated (specified per scenario in table 3).

Drying energy requirements based on removed water		Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6
		Lab Scale, *S. Latissima*	Lab Scale, *U. Rigida*	Lab Scale, Averaged	Upscaled	Upscaled - Short	Upscaled - Simult.
Liquid 1							
Removed water	kg	54	60	57	22	22	0
Drying Energy Ultrafiltration	Wh (!)	31	35	33	13	13	0
Drying Energy Spray drying	kWh	114	128	121	47	47	0
Liquid 1 DM pct	%	3.7%	3.3%	3.5%	8.1%	8.1%	0
Liquid 2							
Removed water	kg	48	60	54	24	24	22
Drying Energy Ultrafiltration	Wh (!)	28	35	32	14	14	13
Drying Energy Spray drying	kWh	102	128	116	52	52	48
Liquid 2 DM pct	%	1.2%	1.0%	1.1%	2.3%	2.3%	10.1%
Solid 2							
Removed water	kg	23	11	16	16	16	16
Oven Drying Energy	kWh	19	9	14	14	14	14
Air Drying Energy	kWh	4.4	2.1	3.1	3.1	3.1	3.1
Solids 2 DM pct	%	6%	12%	8%	8%	8%	8%

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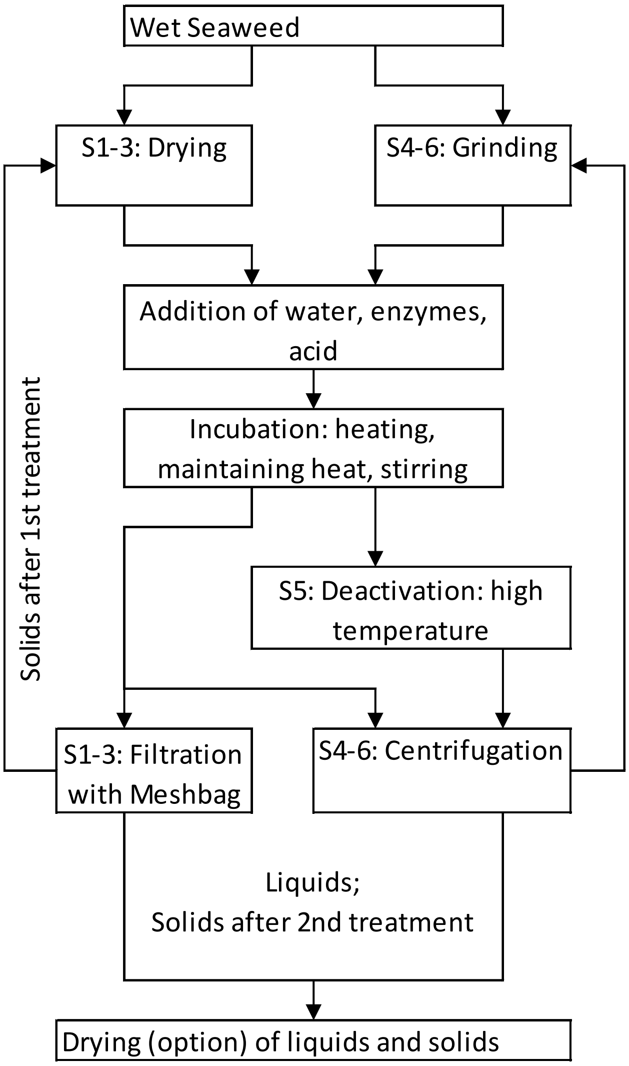
Figure 17: Process steps for the cultivation of enzyme treatment of both seaweeds.



4.1.2.5 Primary data & Inventory modelling

Seaweed inputs, enzyme consumptions and the fractions resulting from the treatments were taken from lab data provided by BDC for *Saccharina latissima* (S1) and *Ulva rigida (S2)*. Lab data from individual treatments runs were averaged to define a single dataset with variables on the distribution for these seaweed species. These datasets were subsequently averaged for S3. The distribution variables were adjusted for the scaled up scenarios S4-6, as well as the enzyme consumption, in interaction with BDC.

Reaction volume was the lab based 80 L for S1-3 with 4-5 kg of dried seaweed (wet mass) added. At the upscaled S4-6, the reaction volume was modelled as 40 L in total with the amount of wet seaweed added corresponding with 4-5 kg dried seaweed, thus operating at the double dry matter concentration. All processes are described at the scale of 4 kg dry matter input, while considering the scale efficiencies of S4-6.

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For consistency in modelling within and between scenarios, full mass balances were constructed. All dry matter and water added to the reaction ends up in the four fractions. Rinsing water was assumed to go to municipal waste treatment. No direct emissions were expected but evaporated water, so none were modelled.

Energy consumption is the key contributor to environmental impacts in processing and has a strong improvement potential and was calculated as follows for different contributions:

- Energy consumption was calculated for air drying wet seaweed for S1-3, the most efficient method of drying, (Slegers et al., 2021) and grinding for S4-6 before enzyme treatment (Piccino, 2016).
- Energy consumption for heating and stirring was taken from lab data for S1-2.
- Heating energy was calculated following Piccino et al. (2016) for S3-6 using reaction volume and time, the heat capacity of water, a scale specific heat loss coefficient and heating efficiency. Stirring was also modelled according to Piccino et al. (2016) for S3-6 considering reaction time and default power number and agitator efficiency and a scale specific impeller diameter and rotational speed. Since the heat loss and stirring apply to the 1000 L vessel with 80 kg of DM seaweed, these were scaled to the reference scale of 100 L with 4 kg of DM seaweed by a factor of 20.
- No energy consumption was modelled for the filtration for S1-3 as it was done manually with a mesh bag, of which the mass was considered in the model.
- Centrifugation and also pumping was modelled according to Piccino et al. (2016) for S4-6 considering the entire reaction volume.

The inputs, outputs, the energy and material consumptions are listed in Table 3 and Table 4 on the next pages.

4.1.2.6 Results analysis

The different contributions to different environmental impacts were mapped for hotspot identification and the choice of one impact category as a lead indicator. The scenarios were compared based on the lead indicator. The impact of individual fractions were also compared in different ways, based on the lead indicator.

The robustness of change from S3 to S4 was studied, focusing on the degrees of freedom for optimization and scale up. The most likely maximum and minimum in Table 7 (see Results) were determined through expert estimates. The spread in enzyme consumptions, temperature differentials and dry matter yields were derived from the lab protocols and data provided by BDC (BDC, 2021). The heat loss coefficient (HLC) was changed for S4 to the HLC of S3 and vice versa, and the relative changes were then used for the minimum and maximum both scenarios. The impeller number and the mesh bag were given a large deviation relative to their certainty to check sensitivity to these variables.

The variation in global warming potential due to the spread in all variables in the enzyme treatment was quantified by calculating impact results from sampled input parameterizations. All variables in the enzyme treatment modelled in the foreground were populated with uncertainty information, in the same informed expert estimation approach as for the variability analysis. Variability in the secondary data was not considered, thus this effort can be seen as a partial Monte Carlo analysis.

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Table 3. Input and output quantities of double enzyme treatment scenarios per (Letters in spread abbreviate distribution type: U = Uniform, T = Triangular, N=Normal, C = calculation, spread derived from input variables).

Input and output quantities		Scenario 1		Scenario 2		Scenario 3		Scenario 4		Scenario 5		Scenario 6	
		Lab Scale, *S. Latissima*		Lab Scale, *U. Rigida*		Lab Scale, Averaged		Upscaled		Upscaled - Short		Upscaled - Simult.	
First enzyme treatment		Value	Spread	Value	Spread	Value	Spread	Value	Spread	Value	Spread	Value	Spread
Seaweed WM	kg	4.30	+/-1% (U)	5.1	4.96-5.24 (T)	4.70	+/-1% (U)	32.07	+/-1% (U)	32.07	+/-1% (U)	n.a.	
Seaweed DM	kg	4.12	4.10-4.14 (T)	4.23	3.97-4.49 (T)	4.17	+/-5% (T)	4.17	+/-5% (T)	4.17	+/-5% (T)		
Water added	L	75	+/-1% (N)	75	+/-1% (N)	75	+/-1% (N)	12.93	(C)	12.93	(C)		
Mass in	kg	79.3	(C)	80.1	(C)	79.7	(C)	45	+/-1% (N)	45	+/-1% (N)		
Solids 1 WM	kg	21.35	15.1 - 27.6 (T)	15.88	13.9 - 17.8 (T)	18.6	16.6 - 20.6 (T)	18.6	16.6 - 20.6 (T)	18.6	16.6 - 20.6 (T)		
Solids 1 DM	kg	2.00	1.60-2.40 (T)	2.09	1.76-2.42 (T)	2.05	+/-5% (T)	2.05	+/-5% (T)	2.05	+/-5% (T)		
Liquid 1 WM	kg	58.0	(C)	64.2	(C)	61.1	(C)	26.4	(C)	26.4	(C)		
Liquid 1 DM mass	kg	2.12	(C)	2.14	(C)	2.13	(C)	2.13	(C)	2.13	(C)		
Second enzyme treatment		Value	Spread	Value	Spread	Value	Spread	Value	Spread	Value	Spread	Value	Spread
Mass in	kg	75	+/-1% (N)	75	+/-1% (N)	75	+/-1% (N)	45	+/-1% (N)	45	+/-1% (N)	45	+/-1% (N)
Solids 1 in DM	kg	2.00	1.60-2.40 (T)	2.09	1.76-2.42 (T)	2.046	+/-5% (T)	2.05	+/-5% (T)	2.05	+/-5% (T)	4.17	+/-5% (T)
Solids 1 in WM	kg	21.35	15.1 - 27.6 (T)	15.88	13.9 - 17.8 (T)	18.62	16.6 - 20.6 (T)	18.62	16.6 - 20.6 (T)	18.62	16.6 - 20.6 (T)	32.07	+/-1% (U)
DM pct in Solids 1 in	%	9.37%	(C)	13.17%	(C)	10.99%	(C)	10.99%	(C)	10.99%	(C)	13.02%	(C)
Water added	L	53.65	(C)	59.12	(C)	56.38	(C)	26.38	(C)	26.38	(C)	12.93	(C)
Solids 2 WM	kg	24.4	(C)	12.3	(C)	17.9	(C)	17.9	(C)	17.9	(C)	17.9	(C)
Solids 2 Yield DM	%	70.6%	52.0-89.2 (T)	68.6%	56.1-81.1 (T)	69.60%	+/-5% (T)	69.60%	+/-5% (T)	69.60%	+/-5% (T)	34.11%	+/-5% (T)
Solids 2 DM	kg	1.41	(C)	1.43	(C)	1.42	(C)	1.42	(C)	1.42	(C)	1.42	(C)
Liquid 2 WM	kg	50.6	(C)	62.7	(C)	57.1	(C)	27.1	(C)	27.1	(C)	27.1	(C)
Liquid 2 DM	kg	0.59	(C)	0.66	(C)	0.62	(C)	0.62	(C)	0.62	(C)	2.75	(C)

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Table 4. Energy and material consumptions of double enzyme treatment scenarios, expressed per ~4.17 kg DM of seaweed treated (specified per scenario in the previous table; Letters in spread abbreviate distribution type: U = Uniform, T = Triangular, N=Normal).

Energy and material consumptions		Scenario 1		Scenario 2		Scenario 3		Scenario 4		Scenario 5		Scenario 6	
		Lab Scale, *S. Latissima*		Lab Scale, *U. Rigida*		Lab Scale, Averaged		Upscaled		Upscaled - Short		Upscaled - Simultaneous	
Energy for drying	kWh	4.9	(C)	5.1	(C)	5.2	(C)	n.a.					
Energy for grinding (dried or wet seaweed)	kWh	0.0516	+/- 50% (U)	0.0612	+/- 50% (U)	0.0564	+/- 50% (U)	0.3848+-/50% (U) for all three scenarios					
Acid for buffer	kg	0.077	+-/50% (U)					0.0133 +-/50% (U) for all three scenarios					
Enzyme added	g	62	0.3-250 (U)					50.5	1-100 (U)	50.5	1-100 (U)	101	2-200 (U)
Temperature differential	degC	n/a	n/a	n/a	n/a	20	+/-20 (T)	20	+/-20 (T)	20+/-20(T) incub./20 deactivation		20	+/-20 (T)
Incubation time	h	n/a	n/a	n/a	n/a	24	n/a	24	n/a	6h incub. / 1h deactivation		24	n/a
Energy for heat	kWh	32	+/-5% (N)	32	+/-5% (N)	3.05 1st Tr / 2.90 2nd Tr. (C)		1.50	(C)	1.42 incub. / 2.80 deactivation		1.50	(C)
Energy for stirring	kWh	6	+/-5% (N)	6	+/-5% (N)	0.093	(C)	0.022	(C)	0.006	(C)	0.00	0.00
Energy for filtration or centrifugation	kWh	0	n/a	0	n/a	0	n/a	0.248	0.019-0.187 (U)	0.248	0.019-0.187 (U)	0.02	(C)
Energy for pumping	kWh	n/a	n/a	n/a	n/a	n/a	n/a	6.9E-04	(C)	6.9E-04	(C)	6.9E-04	(C)
Weight of mesh bag	kg	0.1+/-10% (N) for all three scenarios						n.a. for all three scenarios					
Water added for rinsing (Wastewater)	kg	20	+/-1% (N)	20	+/-1% (N)	20	+/-1% (N)	10	+/-1% (N)	10	+/-1% (N)	10	+/-1% (N)

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4.1.3 *Fucoxanthin extraction*

4.1.3.1 Products and functional unit

Promising results were obtained for the supercritical carbon dioxide extraction (SCE) of fucoxanthin along with other carotenoids from *Saccharina latissima*, at a relatively large lab scale (container size 250 g) at the University of York (University of York, 2021). Moreover, it was found that fucoxanthin has pharmaceutical activity (Pruteanu et al., 2020). The extraction of 1 kg of freeze dried seaweed yields about 6 grams of a fraction consisting predominantly of carotenoids, with a 13 mg/g fucoxanthin content. This extract will be applied as a whole in potential applications, so fucoxanthin is not isolated further. The residue from the extraction will be used for follow up extractions or other processing, but it is not yet known how. The residue might benefit from the treatment with supercritical CO_2 like in the case of (Attard et al., 2015a). For the goal of this study, the environmental impact of the process is of interest, so the functional unit is the provision of 6 g of extract. This corresponds with treating 1 kg of freeze dried seaweed, while 1.05 kg hot air dried and air dried seaweed are required due to slightly lower dry matter contents.

4.1.3.2 Scenarios

Since it was anticipated the drying method influences the environmental impact, three scenarios with different drying options were considered:

1. Freeze drying (lab default, energy demand based on Prosapio et al. (2017), Perez-Lopez et al. (2014)
2. Hot air drying (Van Oirschot et al., 2017)
3. Air drying (similar to the method for drying *Ulva rigida* at ALGAPlus, derived from Slegers et al. (2021)

The SCE process itself can be scaled up by installing more capacity and fitting more extraction cycles in 24 hours without shortening them (Attard et al., 2015b), so scale-up effects will be very small. The SCE process was kept the same across these three scenarios.

4.1.3.3 Process description

SCE is conducted at the University of York according to the protocol from Attard et al. (2015b). Carbon dioxide is heated and pressurized, lead through the substrate and allowed to expand and actively cooled so that the extract remains. No other contributions are there, since carbon dioxide losses are negligible so that the initial consumption of CO_2 should be distributed over a large number of extraction cycles and overall consumption is negligible. The scenarios and process is illustrated in Figure 18.

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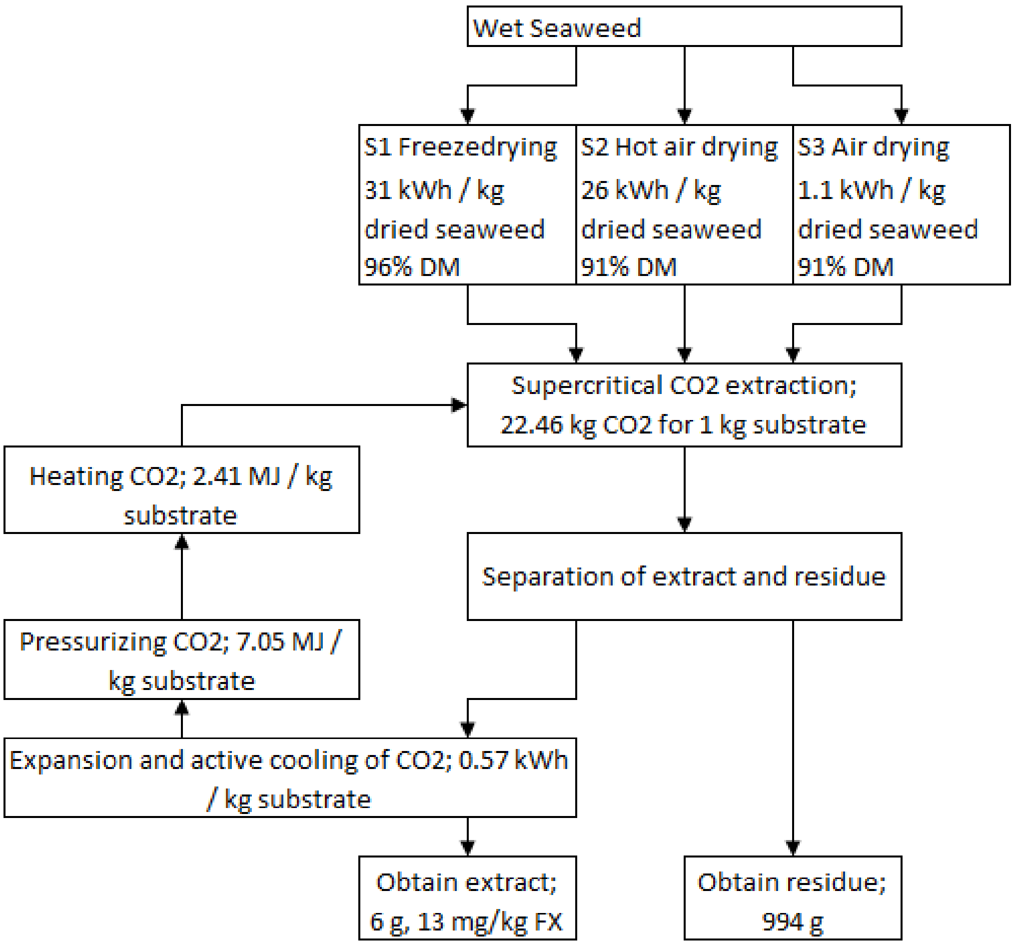
Figure 18: Process description and scenario data for the cultivation of fucoxanthins extraction from Saccharina latissima.



4.1.3.4 Primary data & Inventory modelling

Energy demands from heating, cooling and pressurizing were calculated for a typical extraction cycle, from Attard et a. (2015b). These energy demands were scaled to a 1 kg input and the reported yield of 6 g from University of York (2021) was used as output. The energy demands for drying were derived from a brief literature study for freeze drying. Freeze drying data for strawberries (Prosapio et al., 2017) and for microalgae (Perez-Lopez et al., 2014) were used. Hot air drying was reported by Van Oirschot et al. (2017) for *Saccharina latissima*, but less representative data (Wernet et al., 2016) was used. Slegers et al. (2021) was used to estimate forced air drying energy demands. No direct emissions were expected except for evaporated water, so none were modelled.

4.1.3.5 Results analysis

The different contributions to different environmental impacts were mapped for hotspot identification and the definition of an impact category as a lead indicator. The scenarios were compared based on

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the lead indicator. The effect of variability in input parameters on the global warming potential was quantified for the drying energy demands and the extraction yield by calculating impact results from sampled input parameterizations. There was no reason and no data to assume variability in the SCE process itself. Variability in the secondary data was not considered, thus this effort can be seen as a partial Monte Carlo analysis.

4.1.4 *Secondary data sources*

All data of resources and waste treatment were based on EcoInvent 3.6 (Wernet et al., 2016). SimaPro 9.1 was used for modelling. The impacts of these resources were modelled by selecting the applicable processes listed in Table 5.

Table 5. Secondary data sources for all energy carriers and raw materials provided in Enzyme treatment and Extraction scenarios.

Resource / Waste treatment	Ecoinvent name in SimaPro
Electricity	Electricity, low voltage {Europe without Switzerland}| market group for | Cut-off, S
Heat	Heat, central or small-scale, natural gas {Europe without Switzerland}| market for heat, central or small-scale, natural gas | Cut-off, S
Tap water	Tap water {Europe without Switzerland}| market for | Cut-off, S
Nitric acid	Nitric acid, without water, in 50% solution state {RER}| market for nitric acid, without water, in 50% solution state | Cut-off, S
Enzymes	Enzymes {RER}| enzymes production | Cut-off, S
Mesh bag	Textile, non-woven polyester {GLO}| market for textile, non woven polyester | Cut-off, S
Wastewater treatment	Wastewater from vegetable oil refinery {GLO}| treatment of | Cut-off, S

4.2 *Results*

4.2.1 *Enzyme treatment*

4.2.1.1 Heating and enzymes contribute most to most environmental impacts

The different environmental impacts are shown for S3 Averaged Lab Scale and S4 Upscaled in Table 6 for Liquid2, the focal fraction.

In the case of these scenarios, most of the environmental impacts show similar hotspots. All environmental impacts but freshwater eutrophication have a pattern of contributions that correlate with global warming potential:

- The largest contribution is the first enzyme treatment, in both scenarios, because it is aggregated. The absolute impact of the first enzyme treatment is the same as the second treatment, with the same contributions.

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- Electrical heating contributes most for S3 for most impacts, with enzymes and the mesh bag contributing much too.
- Enzymes (their production) contributes most for S4 for most impacts. Heating is done with natural gas and this contributes to climate change and fossil resource depletion but to a lesser extent to the other environmental impacts. Since the heat demand is lower, the enzyme contribution becomes bigger, compared to S3.

The eutrophication impact indicates high phosphorus emissions from waste water treatment in both scenarios. The global warming potential is chosen as the leading indicator.

Table 6. Impact assessment results for the liquid from the second the enzyme treatment (liquid 2) for scenario 3 Averaged Lab Scale and 4 Upscaled.

Impact category	Unit	S3	S4
Global warming (GW)	kg CO_2 eq	2.66E+00	8.95E-01
Ozone formation - human health (OF-HH)	kg NO_x eq	6.02E-03	1.91E-03
Ozone formation - terrestrial ecosystems (OF-TE)	kg NO_x eq	6.16E-03	1.95E-03
Terrestrial acidification (TA)	kg SO_2 eq	1.24E-02	4.42E-03
Freshwater eutrophication (FE)	kg P eq	4.06E-03	1.47E-03
Marine eutrophication (ME)	kg N eq	9.00E-04	6.18E-04
Mineral resource scarcity (MRS)	kg Cu eq	1.18E-02	3.32E-03
Fossil resource scarcity (FRS)	kg oil eq	7.04E-01	2.38E-01

Figure 19: Contributions to different impacts for scenario 3 Averaged Lab Scale.

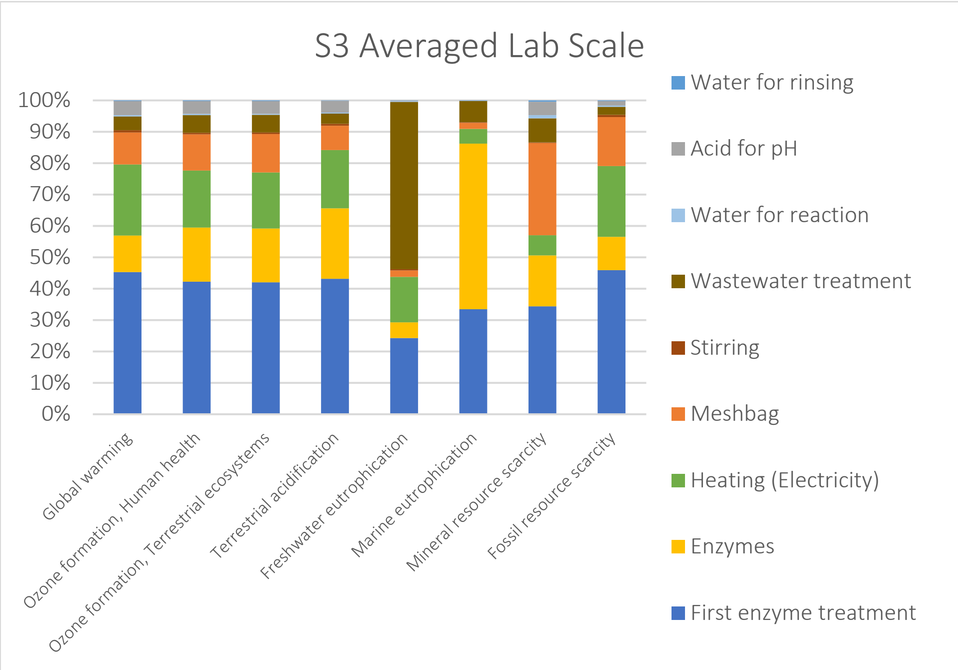

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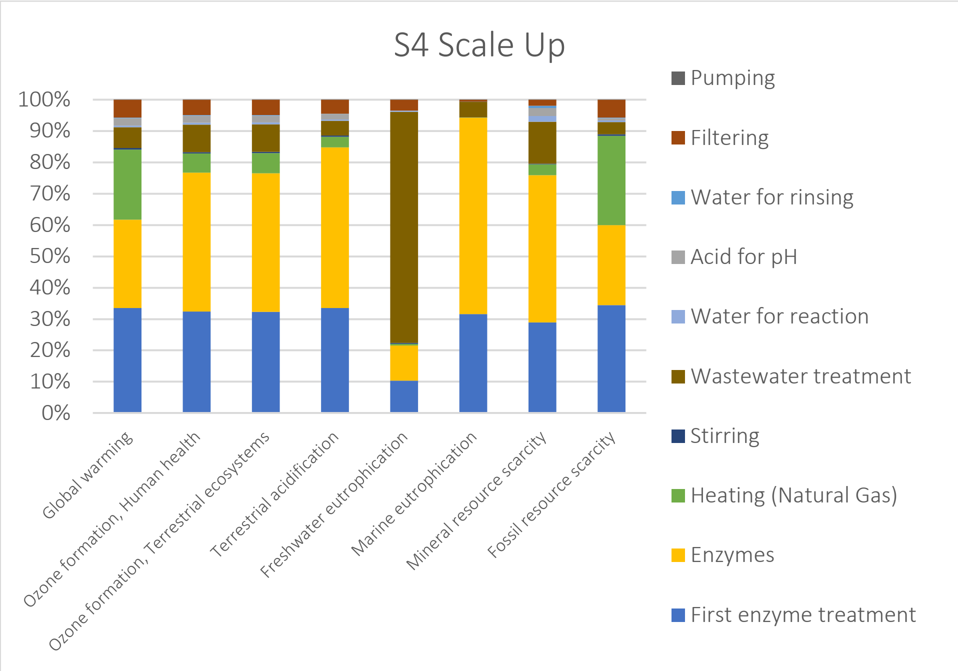
Figure 20: Contributions to different impacts for scenario 4 Upscaled.



4.2.1.2 Heating requirements are significantly reduced upon optimization and scale-up

With global warming potential selected as the leading indicator, it can be determined which resource and energy consumptions contribute most to treating 1 t of seaweed for all scenarios, and the scenarios are compared in Figure 21. Electrical heating on the lab scale is responsible for most global warming potential in the unoptimized scenarios S1 and S2. Stirring is the second contribution for these scenarios. The mesh bag has a small global warming potential for S1 and S2. When moving to scenario S3, heating and stirring are optimized and the energy requirement for drying the seaweed while enzyme treatment becomes a significant contribution along with enzyme and mesh bag consumption. As the energy demand for heating decreases further for the upscaled scenarios S4-6, in different treatment approaches, enzymes take a larger share. Enzymes have approximately the same impact across all scenarios, since their use was lowered slightly from 62 gr in the lab scale (S1-3) to 50 gr in the upscaled scenarios (S4-6).

The potential reduction of global warming potential impact from S1 and S2 to S3 indicates the potential of optimization on the lab scale. The further reduction from S3 to S4-S6 indicates the potential of a higher concentration in the reaction volume. Less reaction volume needs to be heated for the same dry matter amount under treatment. As expected, the temperature increase requires much more energy than compensating the heat losses. The short high temperature deactivation period after incubation in S5 causes a 37% increase in the global warming potential compared to S4.

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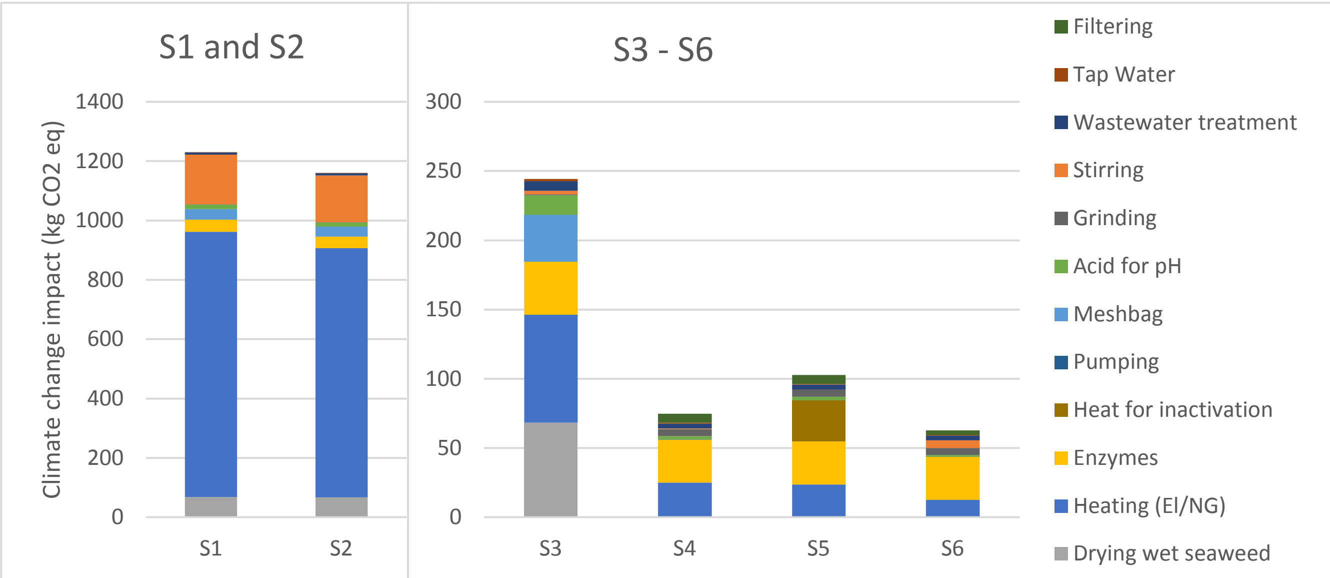
Figure 21: Global warming potential of treating 1 t of seaweed for all six scenarios



4.2.1.3 Impacts of individual fractions follow predictable trends

It was also assessed how the global warming potential is distributed over the different fractions.

The solid from the second treatment receives about half of the global warming potential, since it goes through both enzyme treatments and contains the most dry matter. In S1-5, the two liquids share the other half of the impact equally, which is a mixed effect of the dry matter they contain and energy invested in the fractions. The fractionation does not change under optimization (S3) or scale up (S4 and S5), since the energy demand is the same for both enzyme treatments within a scenario. For S6, all global warming potential is allocated to the only two products that arise. While the enzyme action can be totally different, it was assumed the total amount of dry matter going to the liquids in two treatments ends up in the liquid of the single treatment of S6.

Furthermore, trivial results from a comparison on a dry matter mass basis between different fractions were found. Since less energy is invested in the first treatment, the global warming potential of the liquid and solid from the first treatment are smaller than the second pair of fractions, for all scenarios. S6 only generates one pair of liquid and solid and is the exception. Since the amount of dry matter is used for allocation and for the functional unit, the impact of each treatment is equally shared by liquid and solid resulting from that treatment for all scenarios. Also the trends between the scenarios for individual fractions are identical to the trend in the total like in Figure 21.

4.2.1.4 Anticipated changes in enzyme use and incubation temperature will affect the impacts for both lab and scale-up scenarios

Since the modelling results show that the change in the impact was largest upon scale up, the robustness of this change and the degrees of freedom for optimization were studied for the optimized lab scale scenario S3 and the simplest upscaled scenario S4. The results are shown in Table 7 and in Figure 22. This variability analysis shows that enzymes strongly influence the outcomes (e.g. +/-40% for S4), consistent with its large contribution and its substantial variability.

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The effect of heating is also strong: enzymes operate at optimum temperatures between 20 and 60 °C which result in a variation of about +/-35% in global warming potential. The dry matter yields of both steps have a strong influence on the global warming potential as well, since the effect in the impact (+/-4-5%) is as large as the its variability (+/-5%). A positive change in the dry matter yield of the solid results a negative change in the impact of the liquid. A large change in the heat loss coefficient does not affect the impact strongly, due to its small contribution to total heat demand. This confirms the scaling effect observed in Figure 21 from S3 to S4 is due to higher concentrations. Stirring energy demands are relatively small and do not affect results strongly. The mesh bag use for S3 does affect results strongly either.

Table 7. Variables and results of the variability analysis.

Variable	Input change		Scenario	Output Change	
	Max	Min		Eff. Max	Eff. Min
Enzin	+303%	-99%	S3	52.2%	-17.1%
	+98%	-98%	S4	41.2%	-41.2%
Tdiff	+100%	-100%	S3	34.8%	-34.8%
			S4	33.6%	-33.6%
DMyld	+5%	-5%	S3	-4.8%	5.5%
			S4	-3.2%	3.6%
HLC	+116%	-54%	S3	-3.0%	6.5%
			S4	2.7%	-1.3%
Nimp	+50%	-50%	S3	0.5%	-0.5%
			S4	0.4%	-0.4%
Mesh bag	+10%	-10%	S3	1.5%	-1.5%

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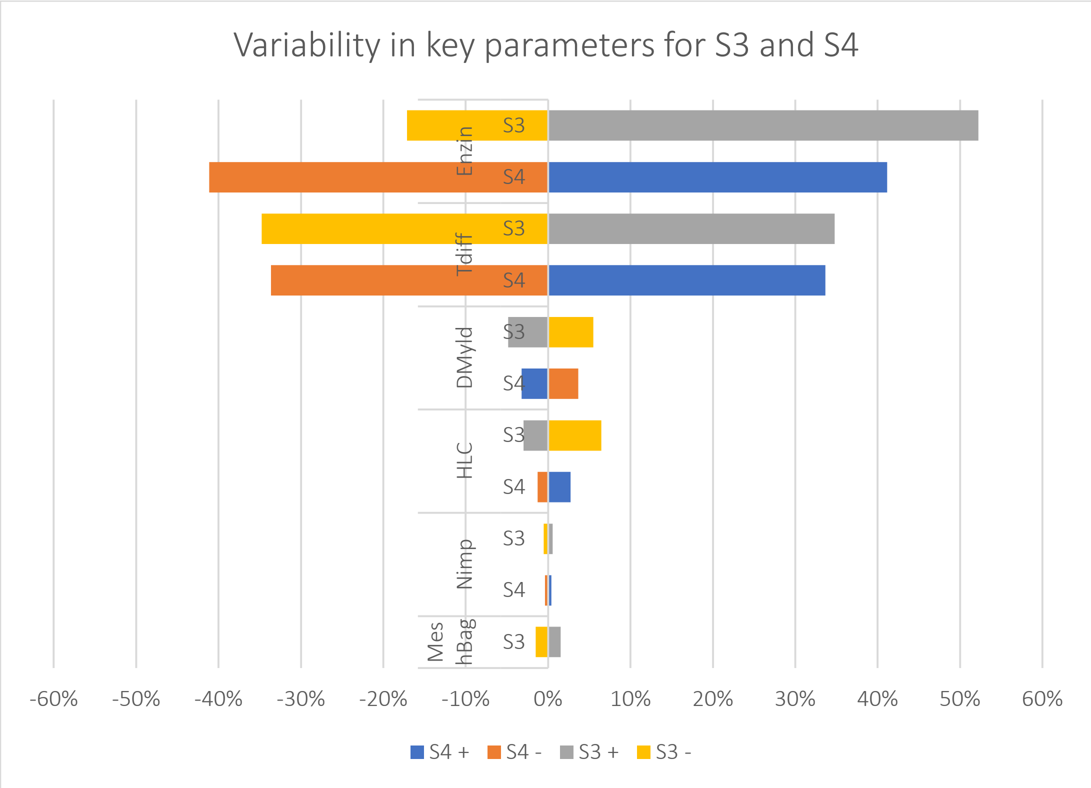
Figure 22: Variability analysis



4.2.1.5 The overall variation in the enzyme treatment is substantial for all scenarios.

The variation is substantial as expected for all scenarios, as illustrated in Table 8 and Figure 23. Variability in most variables drives the variation. While the absolute variation is reduced when moving from S1 to S6 as illustrated with the standard deviation, the relative variation is increasing, as illustrated with the coefficient of variation. A (partial) Monte Carlo analysis on the difference between S3 and S4 indicated that this difference is always positive and at least half (1 kg CO_2 eq) of what is predicted in the deterministic result in 97.5% of the cases. This is because of covariation of many variables in the two scenarios.

Table 8. Variability measures of all enzyme treatment scenarios (SD = Standard deviation, CV = coefficient of variation, 2.5% and 97.5% indicate the percentile values).

	Mean	SD	CV	2.5%	97.5%
S1	14.05	0.979	7	12.33	16.12
S2	13.53	0.817	6	12.04	15.19
S3	3.101	0.6264	20	1.945	4.280
S4	0.869	0.2288	26	0.455	1.281
S5	1.260	0.212	17	0.897	1.625
S6	0.491	0.145	29	0.240	0.743

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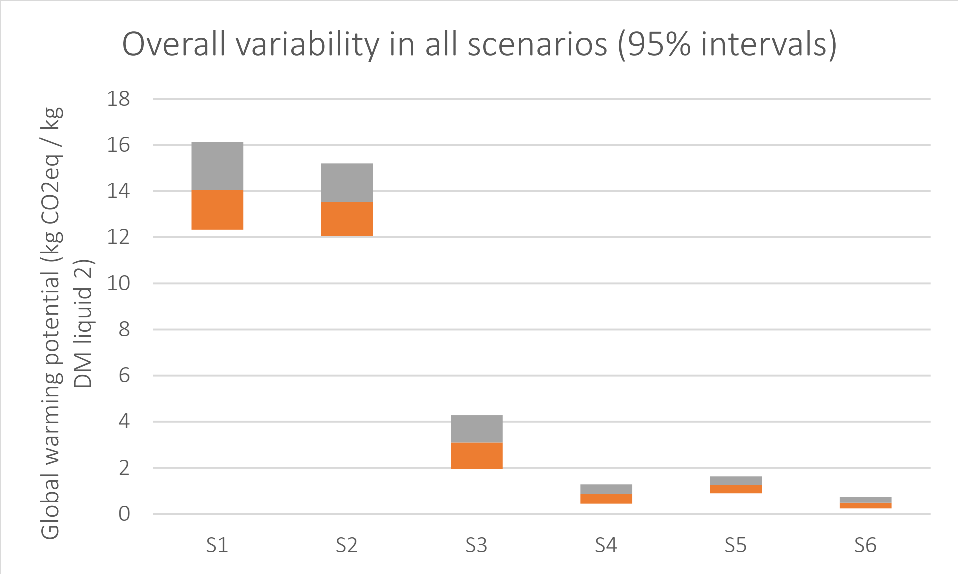
Figure 23: 95 percentile intervals for all enzyme treatment scenarios



4.2.2 *Fucoxanthin extraction*

4.2.2.1 Seaweed drying and CO_2 pressurizing contribute most to all environmental impacts

Different environmental impacts were evaluated for the extraction from air-dried seaweed, as illustrated in Table 9 and Figure 24. The trend of the contributions across the environmental impact is very consistent since energy consumptions (heat and electricity) are the only flows tracked. The drying of the seaweed contributes about 35% while the pressurizing of CO_2 contributes 50–60%. Global warming potential can be selected as leading indicator.

Table 9. Impact assessment results for extraction from air-dried seaweed.

Impact category	Unit	Amount
Global warming (GW)	kg CO_2 eq	1.60E+00
Ozone formation - human health (OF-HH)	kg NO_x eq	2.68E-03
Ozone formation - terrestrial ecosystems (OF-TE)	kg NO_x eq	2.71E-03
Terrestrial acidification (TA)	kg SO_2 eq	5.55E-03
Freshwater eutrophication (FE)	kg P eq	1.39E-03
Marine eutrophication (ME)	kg N eq	9.87E-05
Mineral resource scarcity (MRS)	kg Cu eq	1.90E-03
Fossil resource scarcity (FRS)	kg oil eq	4.37E-01

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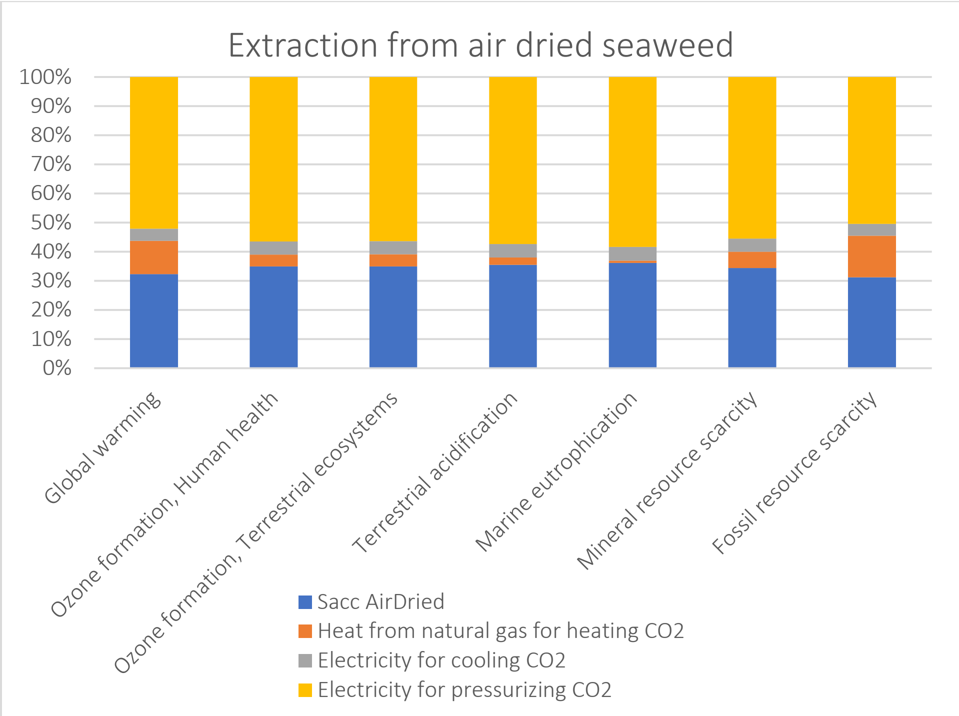
Figure 24: Contributions to different impacts for extraction from air-dried seaweed



4.2.2.2 Variable drying energy demands determine the global warming potential

A comparison of the different drying methods of freeze drying, hot air drying and (forced) air drying is shown in Figure 25. Air drying requires the least energy and results in the lowest global warming potential. For freeze drying, the energy requirement is so high that the impact of the extraction becomes relatively small.

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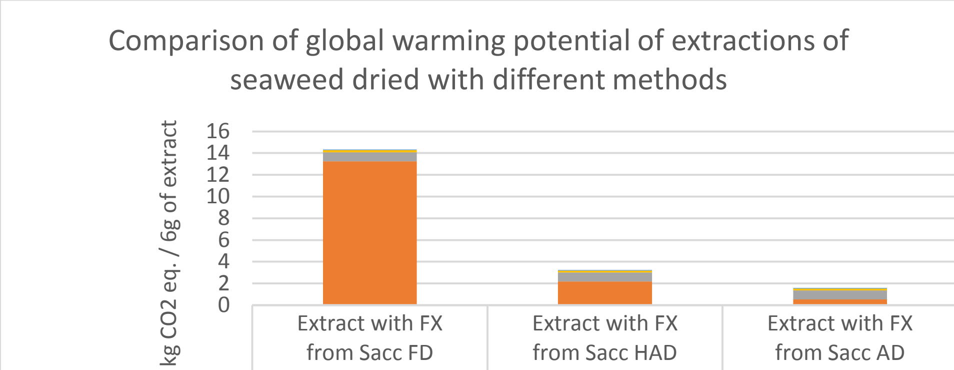
Figure 25: Global warming potential of extractions of seaweed dried with different methods.



	Extract with FX from Sacc FD	Extract with FX from Sacc HAD	Extract with FX from Sacc AD
El cooling CO2	0,07	0,07	0,07
He heating CO2	0,18	0,18	0,18
El pressurizing CO2	0,83	0,83	0,83
El/He drying	13,25	2,17	0,51
El grinding	0,01	0,01	0,01

4.2.2.3 The overall variability in the extraction is substantial for all scenarios

The variation in all three scenarios is substantial due to the uncertainty in the drying energy requirements, as illustrated in Table 10 by the coefficient of variation (CV) and in Figure 26. The CV for the hot air drying scenario is largest.

Table 10. Variability measures of all extraction scenarios (FD = Freeze drying, HAD= Hot air drying, AD = air drying, SD = Standard deviation, CV = coefficient of variation, 2.5% and 97.5% indicate the percentile values).

	Mean	SD	CV	2.5%	97.5%
1 FD	14.3	1.4	9.7	11.8	17.3
2 HAD	3.2	0.48	15.1	2.4	4.2
3 AD	1.6	0.18	11.3	1.3	2.0

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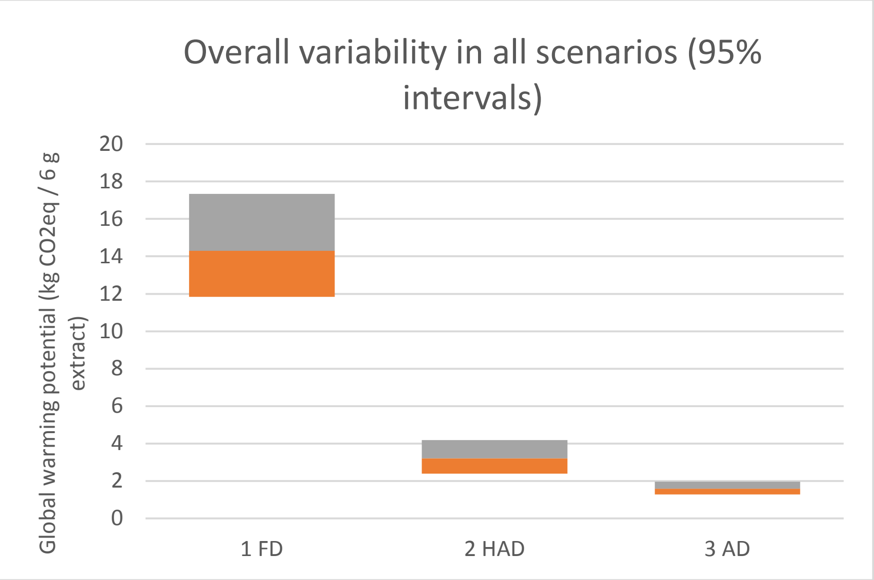
Figure 26: 95 percentile intervals for all extraction scenarios.



4.3 Discussion

4.3.1 Enzyme treatments

4.3.1.1 Variability in energy demand determines scenario differences and hotspots

In the LCA of different enzyme treatment scenarios, a clear variation within and across scenario results was observed. Different hotspots were identified across the different scenarios and the identification is most likely robust. Energy demands for heating contribute much across all scenarios. When moving from lab scale to scale-up, manual filtration with mesh bags is replaced with electricity consuming filtration and the heat required for drying is eliminated. Moreover, energy demand for heating is halved thanks to a doubling of the concentration in the reaction.

The focused variability analysis in 4.2.1.4 and overall variability analysis in 4.2.1.5 indicate the trends in global warming potential and its hotspots remain the same across the scenarios when considering the potential input variability in primary data. It is expected the variation in the results is caused by strong variability and uncertainty in practice and to a lesser extent by uncertainty in the assessment. It is challenging and laborious to identify unknown uncertainties and to systematically assess uncertainties in the secondary data.

The estimation of the heat demands for all scenarios is quite robust, although Piccino et al. (2016) is a very dominant source for these estimations. Bieler (2004) proposed two frameworks (top down and bottom up) for estimating energy consumptions for chemical processing a decade earlier, but these were not found suitable. The bottom-up framework was to detailed and relies much on field measurements. These are not possible since the enzyme treatment does not exist at the large scale. The top down framework indicates electricity demands but the current approach is not compatible with Bieler's (2004) approach:

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- Electricity demand is determined for total operation and process cooling and steam is determined for process and building heating in Bieler (2004) whereas in this study demands for heating from gas and electricity are calculated for a set of unit operations.
- The plants addressed with reliable regressions in Bieler (2004) are a multiproduct and a monoproduct organic batch plant with operating temperatures of -20 °C to +250 °C in whereas the current study is about operations based on water as solvent.
- The lowest capacity from the regression dataset from Bieler (2004) is 1100 ton/month. In comparison, if the batch size of the enzyme treatment of 10 m3 (Olmix, 2021) would be utilized two times a day, 600 ton/month of seaweed could be processed and the output level would be 925 ton/month, but it would include mostly water.

For all scenarios, a major uncertain factor is the very large contribution of wastewater treatment to freshwater eutrophication is large. This contribution comes from the secondary data from waste water treatment. It is not likely the enzyme treatments feed large amounts of phosphorus to the waste water treatment, so it should be modelled better.

4.3.1.2 Enzyme impact is uncertain but will be reduced in practice

The enzymes contribute exceptionally much for marine eutrophication, which indicate high nitrogen emissions in the production of enzymes. This might be correct and should be studied better. The enzymes also contribute unexpectedly much to global warming potential for the scaled-up scenarios. BDC is currently improving enzyme usages in cooperation with Olmix (2021). The estimated maximum usages from the variability analysis have occurred for some enzyme types in the past and BDC is moving toward the estimated minimum for current enzyme treatments in the lab. On the industrial scale, the minimum usage is also very likely. The environmental impact of enzymes could be modelled better than with a single source of secondary data, and type and quantity of enzymes should be considered better, but their significance to the environmental impacts will decrease.

4.3.1.3 Water contents: technical and methodological uncertainty

Products from enzyme treatment inevitably contain substantial amounts of water. Each solid will at least have the high water matter content of the native seaweed and each liquid is even less concentrated. The drying energy requirements are highly uncertain, since a lack of clear literature sources on concentrating such fractions. It may or may not strongly influence the global warming potential of the fractions. For example, in S4 roughly 3.4 kWh of energy is used for treating 4.2 kg if dry matter seaweed, while the resulting 27.1 kg wet mass of liquid with 2.3% dry matter requires 52 kWh of heat for spray drying but only 0.014 kWh for ultrafiltration to concentrate to about 2.6 kg of WM. The energy demands for drying solids are smaller and less variable. All heating requirements are in listed in Table 2 (Methodology: Enzymatic treatment.) Drying is however not mandatory, since other downstream processing could be done in water, and final products can contain some water. Since the fate of the water in the product was uncertain, it was kept out of scope.

4.3.1.4 The scale up should focus on yields, concentration and temperature differential

The partial Monte Carlo analysis indicated that the relative uncertainty is increasing from S1 to S6. This is an expected effect when estimating scaling up and considering future processing options (S4-S6) while a process is still operated on the lab scale. However, the differences in impact are large enough that the effects of different scenarios are robust.

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The strong influence of the temperature differential indicates an improvement potential during process optimization, during scale up and for future environmental assessments. The heating energy demand is also influenced by the reaction volume. The increase of dry matter concentration from approximately 6% on the lab scale to 10% in the upscaled scenario is challenging at the industrial scale. The concentration is limited by grinding step, in which the addition of water is required (Olmix, 2021).

The effect of increasing the vessel size was very limited, but scale-up by an additional factor of ten should be studied. Stirring may require more energy because a second moving part in the vessel is required to scrape seaweed particles during enzyme incubation. Moreover, electrical heating on the lab scale and gas heating on the scaled up level will be replaced by hot water or steam heating. This change of heat source will affect absolute global warming potential by some percents and will not change the conclusions.

Project partners currently focus on fixing the temperature differential and prefer a single enzyme treatment (or simultaneous enzyme treatment). Moreover, the energy demand of the short high temperature deactivation period after enzyme incubation adds much to the enzyme treatment's global warming potential. Single enzyme treatment will also affect the yields, and the impact results will become better known and fall within the estimated variability ranges. During this stage of technical development, technical and economic considerations determine choices on temperature and enzyme use of the innovation more strongly than the aim for a lower environmental impact. Since reducing energy and enzyme use reduces costs, economic and environmental considerations coincide in this case.

4.3.2 *Fucoxanthin extraction*

Thanks to the focus on energy consumption from the predictable supercritical carbon dioxide extraction (SCE), the results of the fucoxanthin extraction are robust and easy to interpret. Energy contributions drive all different environmental impacts and the different seaweed drying methods before extraction drive the differences between the scenarios. The data sources for drying are very different, and quite uncertain. Direct measurements on industrial and lab scale for different drying methods are recommended. The overall variability analysis shows the trends are robust despite the limitations.

Both drying and extraction activities are high energy investments, yielding a pharmaceutically active ingredient of high value. Perhaps the residue also benefits from CO_2 treatment but little is known regarding this. Further integration into a production chain after further research of the residue is required. Next, an environmental assessment with an expanded goal and scope could shed more light on the balance between environmental impact and functional value of the different products.

The fucoxanthin and the other pigments in the extract are sensitive to oxygen and light. They should be protected from these conditions in the production chain, as soon as the seaweed is harvested. While freeze drying is quick and oxygen free it requires a lot of energy, other methods could be adapted to ensure the mild conditions. Forced air drying already takes place in dark chambers and a forced nitrogen flow could be considered.

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4.3.3 *Outlook*

4.3.3.1 The results are of sufficient quality to address goals of the LCA

The energy oriented assessment of supercritical carbon dioxide extraction and the more complex assessment of different enzyme treatments are sufficiently robust to draw conclusions about the study goals. The robustness of the results was ensured for both processing options by including energy, fuels, water and auxiliary materials and by addressing known uncertainties. Hotspots have been identified and differences between scenarios were explained, and this information was used to provide recommendations or focal points improvement of both processing options.

4.3.3.2 Knowledge about product applications will aid interpretation and optimization

The overall global warming potential ranges from 0.063 (S6) to 1.2 kg CO_2 eq/kg seaweed treated (for S1 and S2) for enzyme treatment and from 1.6 to 14.3 kg CO_2/kg seaweed treated for SCE. Whether this is high or low depends on how these products are applied. Once applications are better known, comparisons can be made with benchmarks, and trade-offs in yield or quality between two fractions from the same treatment can be identified. Also, the question how to attribute environmental impact from a chemical separation to the two resulting fractions (solid/liquid or extract/residue) can be addressed better.

Drying is an energy investment, mandatory (and included) for SCE and optional (and excluded) for enzyme treatment. In any case, this investment should be justified by the benefits it yields in terms of processing or in valuable products. A high water concentration also affects the economic viability and environmental impact of transporting the products. In this justification, technical and economic factors play a role in addition to environmental considerations. It is recommended to avoid thermal drying methods and limit water additions to the reaction mixture and to locate the future supply chain close to the source of the feedstock.

4.3.3.3 Future LCAs should and can have a broader scope

During scale-up, new technical insights will reduce the known uncertainties and elicit new uncertainties. Furthermore, energy requirements will probably decline and other contributions to environmental impacts may emerge. SCE could be for example a process with intensive capital goods usage. In the future, an LCA with a broader scope will become feasible and informative. For example, an entire production plant or a supply chain can be assessed and optimized, considering new trade-offs. The insights derived from this LCA serve as building blocks for future LCAs.

4.4 Conclusion

The goal of part II of this report is to map the environmental impact of individual or combined processing steps and identify hotspots within the steps for enzyme treatment and supercritical carbon dioxide extraction (SCE). In general, energy demands contribute strongly to the results and global warming potential can be a lead indicator for all environmental impact categories. When considering uncertainty in both assessments, the trends in the results and the obtained insights remain the same.

In the LCA of the enzyme treatment, it was found that heating and enzymes contribute most to different environmental impacts. Upon scale-up, heating demand significantly reduced in the scenarios thanks to a doubling of the concentration of seaweed in the reaction volume. In practice, increase in concentration is expected along with a much lower enzyme use. The expected variability in

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temperature differential and enzyme use strongly affect the global warming potential. These factors are focal points for optimization and scale-up, together with yields and reactant concentration.

In the LCA of SCE, it was found that seaweed drying and electricity use for pressurizing CO_2 during SCE contribute most to different environmental impacts. The energy required differs strongly for the three studied seaweed drying options. It is expected scale-up will not change this, but a trade-off between energy efficiency and mild conditions should be found for the drying method.

The LCA of these seaweed processing methods provide interesting recommendations for the further technical development process. The points of attention for scale-up and optimization are not highly surprising, nor would environmental considerations dominate choices during technical development. In the current stage of development they coincide with economic considerations. However, these results quantify the potential for improvement of the environmental impacts during the current phase of innovation and set clear priorities. In the future, more will be known about the applications of these products and this will aid optimization further and questions regarding a future supply chain strategy can be addressed. The work on data and methods in the current study, especially the consideration of uncertainty, will aid future LCAs of these applications and prospective LCAs on other seaweed applications under development.

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