



# Life cycle assessment of heterotrophic algae omega-3

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## ABSTRACT

Fish oil has traditionally been the primary source of long chain omega-3 fatty acids, which are essential nutrients for human diets as well as many aquaculture and animal feeds. The demand for fish oil is growing rapidly, due to an expanding aquaculture sector as well as rising demand in pet and livestock feeds, while the availability of fish oil from wild caught fish has leveled off over the past decade. Fish oil is not easily replaced and alternative sources are required to meet the growing global demand. One of the most promising alternatives is microalgae - the original source of long chain omega-3s. To understand the environmental impacts of omega-3s produced by heterotrophic algae, a comprehensive Life Cycle Assessment (LCA), assessing six impact categories, was conducted for two algae omega-3 DHA (docosahexaenoic acid) products, in powder and liquid suspension formats. These products are manufactured at industrial scale using sugarcane for both feedstock and a renewable energy source. The life cycle impact assessment results for algae omega-3 DHA indicate that sugarcane cultivation has the largest contribution for most of the categories. A sensitivity analysis revealed that the sugarcane yield, and the sugar to omega-3 DHA yield, were the most relevant parameters, and that the choice of allocation methodology did not have a strong influence on the results. A comparison with fish oil, using data publicly available in LCA databases, indicated that the two formats of the commercial algae omega-3 DHA product offer about 30–40% lower impact on climate change than fish oil.

## 1. Introduction

Long chain omega-3 fatty acids—eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids—are essential for human diets as well as many aquaculture and animal feeds. The developmental and health benefits of long chain omega-3s for brain, eye and heart health for humans is well documented [1]. Many of these same benefits are important for animal development and growth as well.

Fish oil has traditionally been the primary source of long chain omega-3s, but is sourced from finite marine fisheries, and supply has leveled off at around 1.1 million tons in recent years [2]. A recent study tracked flows of long chain omega-3 stocks through global production, supply and utilization pathways and found a significant gap in supply [3]. This supply gap will continue to widen as demand rises, especially from the aquaculture sector which consumes approximately 75% of the available crude fish oil [4]. Aquaculture is one of the fastest growing food systems in the world, with fed species representing nearly 70% of global aquaculture production [5]. Aquafeed, especially for carnivorous species, historically relied on forage fish for fish meal and fish oil, to

provide the required nutrients such as protein and omega-3 fatty acids. With the global supply of forage fish at a plateau, there has been rising demand to limit the use of wild marine fish in aquaculture feed in order to reduce pressure on already stressed wild fisheries [6]. A recent study of salmon feed in Norway showed that over 70% of aquafeed is now sourced from plant origins [7]. For example, fish meal was the original source of protein in aquafeed and now is partially being replaced by soy protein [7]. Novel ingredients such as insect meal and protein derived from single cell bacteria are emerging as alternative protein sources to fish meal and soy [8].

In an effort to reduce the use of fish oil in aquafeed, some fish oil has been replaced by rapeseed or camelina oil which meets the energetic needs of farmed fish, but not the omega-3 needs [7]. Dropping levels of long chain omega-3s in carnivorous fish, especially salmon, can impact fish health and welfare [9]. In addition, the change in nutritional quality of salmon, an increasingly popular species in the global market, can impact public health due to less intake of long chain omega-3s [10].

Fish oil contains key long chain omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid and docosahexaenoic which are not

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easily replaced. Therefore, alternative sources are required to meet the growing demands, not only of aquaculture, but also livestock production and direct human consumption [11]. Thus, scientists have looked to the original source of long chain omega-3 PUFAs – microalgae – as a potential solution.

Heterotrophic microalgae from genera such as *Schizochytrium*, *Ulkenia*, and *Cryptocodinium* produce algae products that are rich in DHA [12]. Algae from these genera have been selected based on their ability to produce large quantities of DHA from a carbon feedstock, through conventional fermentation.

Corbion has developed and commercializes omega-3 DHA products which utilize an algae of the *Schizochytrium* genus to produce biomass that is rich in DHA and is applied as an alternative to fish oil in feed for aquaculture and livestock, as well as pet food. This heterotrophic microalgae product is manufactured via fermentation in Orindiúva, Brazil, using sugar from sugarcane as feedstock. The production site is co-located with a sugar mill enabling the efficient use of energy from sugarcane by-products. This novel omega-3 DHA source shows promise for reducing the amount of wild fish needed in feed for farmed fish and livestock, as well as pet food, while maintaining or improving animal health.

A comprehensive understanding of the environmental impacts of algae feed ingredients is lacking [8]. Although algae-based ingredients reduce the demand of wild fish, other environmental impacts associated with the production of these algae ingredients must be evaluated. The potential environmental trade-offs need to be well understood to ensure the sustainability of algae-based omega-3 production. Life cycle assessment (LCA) is a well-established methodology that uses a holistic approach to identify trade-offs between environmental impacts to avoid shifting burdens over the value chain. Due to characteristics such as fast growth, high cell density, and high oil productivity, heterotrophic algae systems are of increasing interest [13], however, to our knowledge, there are no LCAs available in the public domain about commercialized heterotrophic algae systems [13–15]. The literature studies on the LCA of heterotrophic algae systems focus on biodiesel production. Other LCA's on feed and food production of protein and oil ingredients are based on lab or pilot data [14–16].

This paper aims at providing cradle-to-gate LCA information on commercially produced omega-3 DHA from heterotrophically grown microalgae to scientists, feed producers, and aquaculture and animal farmers, who seek to understand the environmental sustainability aspects of heterogeneous algae products. The novelty of this LCA study is the use of industrial data for algae omega-3 production. Additionally, the LCA results are compared with the environmental impact of producing omega-3s from fish oil. The commercial production of omega-3 DHA via heterotrophic microalgae has optimization potential that can be realized through continuous product and process development. In this study, the impact of some of these improvements are quantified to provide an outlook of the potential of algae-based products.

## 2. Materials and methods

This LCA was performed according to the standard methodology described in ISO 14040 series by the International Organization of Standardization [17,18]. The LCA model was created in SimaPro developer software version 9.1 (PRé Sustainability, The Netherlands).

### 2.1. Goal and scope definition

The goal of this LCA is to quantify the environmental footprint of omega-3 DHA produced from heterotrophically grown microalgae, commercialized under the name of AlgaPrime™ DHA. The results are intended to be used as input in the LCA of feed formulations and to understand the environmental sustainability of the fish oil alternative for omega-3s. The study relied on an attributional LCA approach, therefore the potential impact of feed and food system transformation

due to heterotrophic microalgae production was not considered.

The functional unit was defined as *1 kg omega-3 fatty acids*. Although other components of the algae biomass, such as proteins, carbohydrates, and other fatty acids, have nutritional value for feed, omega-3s are the reason for inclusion of the algae product in feed. For this reason, the choice of the functional unit reflects the primary function of the algae omega-3 DHA product. Furthermore, this functional unit allows comparison between the algae omega-3 products and fish oil. In the case of the algae product studied, DHA is the only long chain omega-3 fatty acid, whereas in the case of fish oil, both EPA and DHA are present. Traditionally, aquafeed contains varying ratios of EPA and DHA depending on the type and amount of fish oil included. In a study conducted on salmon, it was shown that omega-3s are essential to the growth and health of the fish and when omega-3s were excluded from the diet entirely, the fish growth rate was slower and overall health of the fish decreased [9]. DHA appears to be the more essential of the two PUFAs and has been suggested that the long chain PUFA requirement of healthy salmon can be met by DHA alone [19]. Consistent with previous research, a recent study found that DHA has higher omega-3 retention in the filet of salmon versus EPA, which is typically metabolized by the fish [20]. In additional studies by Nofima, one of the leading aquaculture research institutes in Norway, it was determined that DHA rich *Schizochytrium* microalgae effectively replaces fish oil in diets of Atlantic salmon in both freshwater and sea-water life stages [21].

The omega-3 DHA algae is produced in two feed ingredient formats - powder and liquid suspension - to offer flexibility for feed producers. A diversity of feed formulation formats is needed for aquaculture species, livestock, and pets due to consumption patterns, digestive and absorption dynamics, and operational application needs. Both formats are included in the scope of this study:

- 1) Powder or biomass - Oil high in omega-3 DHA is encapsulated within the algae cell to form a free-flowing dry powder.
- 2) Liquid suspension - Blend of omega-3 DHA-rich algae and vegetable oil.

Hereafter, we will refer to these products as “algae omega-3 DHA powder” and “algae omega-3 DHA liquid suspension”. Both products have a similar DHA content of 28–30 wt%.

The scope of this LCA is cradle-to-gate and includes (1) sugarcane cultivation and harvest, (2) processing sugarcane to sugar (with energy and ethanol as co-products) and (3) conversion of sugar into algae omega-3 DHA by Corbion. The production of the algae omega-3 DHA products is located at a Corbion facility in Brazil where current production is ongoing. The input data for production used in this study is based on calculations for full scale production, using techno-economic models and manufacturing production data from 2019. Manufacturing of production equipment, buildings, and other capital goods on the manufacturing site of Corbion are not included in the scope. Due to the long lifetime of the plant, the contributions are expected to be small.

### 2.2. Environmental impact categories

The characterization method used was the EF (Environmental footprint) 3.0 impact assessment method [20] adapted to SimaPro 9.1. This is the impact method published for use during transition phase of the Environmental Footprint initiative [22]. The study covers the six most relevant impact categories defined by the product environmental footprint guidelines category rules (PEFCR) for animal feed [23]: (1) Climate change (GWP100a based on the Intergovernmental Panel on Climate Change - IPCC, 2013 [24]), (2) Particulate matter (impact on human health [25]), (3) Acidification of terrestrial and freshwater (Accumulated Exceedance [26]), (4) Land Use - LANCA model [27], (5) Terrestrial Eutrophication (Accumulated Exceedance [26]) and (6) Water use - AWARE model [25].

### 2.2.1. Biogenic carbon

Sugar used for algae omega-3 DHA production contains biogenic carbon which is stored in the plant tissue during plant growth and converted to omega-3 DHA. In this LCA, biogenic carbon does not earn a credit for carbon uptake because most of the carbon will eventually be released again as CO<sub>2</sub> or CH<sub>4</sub> after animal or human consumption in less than a few years. This means that the carbon uptake of the crop during cultivation is not considered and neither are the CO<sub>2</sub> emissions after consumption. This approach is consistent with the PEF (Product environmental footprint) guidelines [28].

### 2.2.2. Additional information

As additional information, the water scarcity risk was assessed using a water risk assessment tool - Aqueduct Water Risk atlas [29].

## 3. Process description and life cycle inventory

The production of algae omega-3 DHA has five main steps: (1) cultivation of sugarcane and transportation to sugar mill, (2) processing of sugarcane into multiple products: sugar, ethanol, and electricity, (3) transformation of sugar by algae into DHA via fermentation and (4) downstream processing of algae into the final products. The production system can be seen in Fig. 1. Below is a detailed description of each step.

Primary data for algae omega-3 DHA production and downstream processing (see the system boundary line of “Corbion” in Fig. 1) was obtained from the engineering model (Corbion engineering team in Orindiúva, Brazil). The model is based on kinetic models for the fermentation validated at commercial scale, thermodynamics and mass and energy balances for the existing process and equipment. These data sources are chosen to ensure the consistency and comparability between both formats of the products. For the main inputs, sugar, electricity and steam, primary data was collected from the actual suppliers. Background data were obtained from LCI (Life cycle inventory) databases and modified if necessary, as described in the next sections. For the agricultural products, the Agri-footprint database (Blonk Consultants, The Netherlands) was used and partially modified (e.g., sugarcane cultivation, see Section 3.1) to reflect the farm and sugar mill-specific data from the suppliers. For all other background processes, Ecoinvent v3.6 APOS was used (Ecoinvent, Switzerland).

The aggregated LCI for algae omega-3 DHA powder and liquid suspension is provided as supplementary information both as \*.csv file for SimaPro 9.1 - Supplementary material.

### 3.1. Sugarcane production

Production of algae omega-3 DHA uses sugarcane sugar from Brazil as feedstock. Brazil is the world's leading producer of sugarcane with 41% of the global production in 2017 [5,30]. Sugar is supplied by a sugar mill located adjacent to the Corbion facility. This mill, located in the state of São Paulo, uses sugarcane from the surrounding fields and has been in operation for over 20 years.

Sugarcane production consists of land preparation, planting of cuttings, application of synthetic and organic fertilizers and plant protection agents, maintenance of the crop and harvesting. The organic fertilizers consist of vinasse and filter cake which are byproducts of sugar and ethanol production. The application of fertilizers to the fields have become a focus of many studies in attempting to reduce the environmental footprint of sugarcane [31,32]. In this region, the sugarcane fields are primarily rain-fed, with application of the mill by-products and returned treated water from the mill. This irrigation technique was shown to increase the crop yields [33].

Harvesting of the sugarcane is mechanized and burning cane residues is prohibited by the local legislation [33]. The prohibition to burn cane residue in open air has led to a major decrease in CO<sub>2</sub> emissions, decrease in particulate matter and improvements in human health. Currently, the crop residues (tops and leaves) are left in the fields after

harvesting, but there is on-going research to understand the best practices for the management of residues, aiming at reducing emissions, mostly N<sub>2</sub>O, from crop residues [32].

The inventory for sugarcane production was modelled by modifying the Agri-footprint v4 unit process of “Sugar cane, at farm/BR Energy” with available primary data provided by the supplier, to make the LCI input site-specific. The dataset modifications are summarized in Table 1. Sugarcane production is not allocated. After harvesting, the entire cane is transported to the sugar mill. The sugarcane residues left on the field are not considered as by-products and the emissions related to the residues left on the field are included in the LCI.

Direct land use change (DLUC) refers to the change or conversion of the original land use (forest, grassland, pastureland, etc.) to another land use, i.e. sugarcane plantations. Depending on the type of conversion that occurs, DLUC can unlock carbon that is stored in soil and vegetation, which is released as CO<sub>2</sub>. The total GHG (greenhouse gas) emissions and removals arising from DLUC over a period of 20 years must be included in the quantification of GHG emissions. Knowledge of the prior land use may be demonstrated, for example, by using satellite imaging to identify and measure historical land use change. With this purpose, GRAS (Global Risk Assessment Services GmbH<sup>1</sup>) conducted a study for Corbion based on high-resolution satellite images and enhanced vegetation index time series analysis over the period between 1999 and 2019, covering the areas of the sourcing plantations of the sugar mill. The results showed that, for the sourcing area of the mill, the only significant land transformation was from degraded pastureland to sugarcane (48%) [34]. The GHG emissions from DLUC were calculated based on the measured transformation areas and types of land transformation [35]. The conversion of degraded pastureland with the lowest biomass carbon stock to sugarcane cultivation can potentially be a carbon sink, depending on the soil types and agricultural management practices, resulting in negative DLUC [33]. In order to make a conservative estimate, a DLUC value of zero kgCO<sub>2</sub>eq/t sugarcane was used.

The second component of land use change is indirect land use change (iLUC), which considers secondary effects induced by large-scale expansion. The displacement of existing crops potentially leads to the expansion of cropland elsewhere. There are different models used to assess iLUC with no consensus yet on iLUC methodology [36]. In the case of Brazilian sugarcane, most of the land displacement occurring is related to conversion of pasture lands for cattle into sugarcane fields enabled by livestock intensification. Additionally, improvements in agricultural yield and expansion of sugarcane into regions with higher potential for agricultural productivity are pointed out as explanations for the low iLUC impact related to sugarcane in the region of São Paulo [33,36,37]. iLUC is excluded from this study to reflect the attributional nature of this study and also in alignment with the PEF and the PEF CR guidance [22,23].

Fertilizers and direct emissions from fertilizer application are key aspects for the LCI of agricultural products. Specific information provided by the supplier was used to modify the original Agri-footprint dataset:

- The amount of fertilizers was adjusted based on the total values of NPK (kg N, P and K/ha) provided by the supplier.
- The emissions from vinasse and filter cake application were calculated based on supplier information regarding the amounts and composition, based on Macedo et al. 2018 [38]. The IPCC methodology was applied, consistently with the Agri-footprint methodology [39].

### 3.2. Sugar mill

After harvest, the cane is transported by truck to the mill and

<sup>1</sup> <https://www.gras-system.org/>.

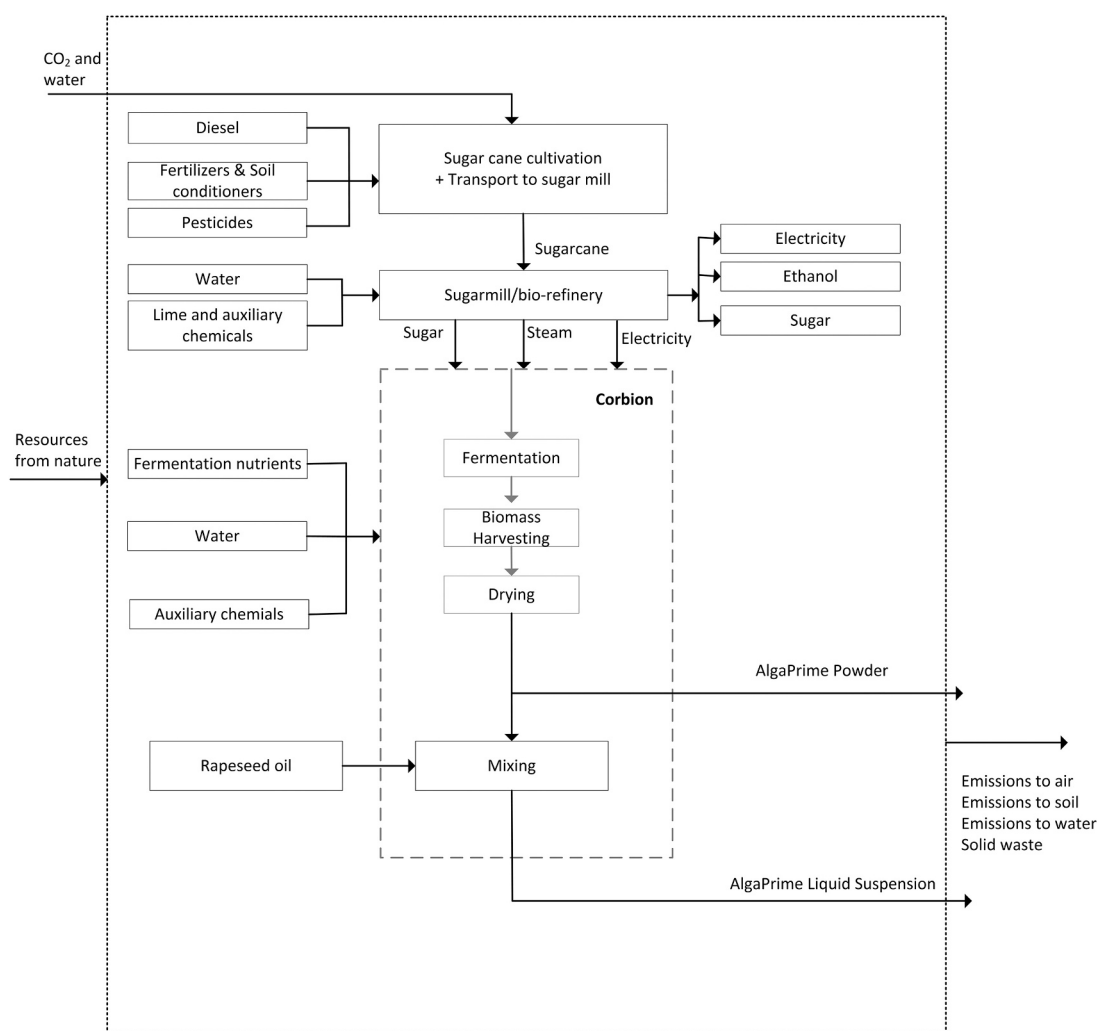


Fig. 1. Production system of algae omega-3 DHA (docosahexaenoic acid) considered for the life cycle assessment study.

Table 1

Overview of the modifications to the Agri-footprint sugarcane dataset, to reflect the supplier specific information. DLUC – direct land use change.

| Parameter  | Modification  | Reference   |
|--|---|---|
| DLUC   | Zero CO <sub>2</sub> emissions from DLUC are considered based on the results of satellite imagery between 1999 and 2019 | (GRAS, Global Risk Assessment Services, 2019) [34]  |
| Amount of fertilizers and direct emissions from fertilizer application | Described in the text   | Supplier information (confidential)   |
| Irrigation   | No water input from natural sources. Only effluent from the sugar mill, filter cake and vinasse are applied             | Supplier information  |
| Harvesting   | All mechanized harvest  | Supplier information (FAO, 2020 [30])   |
| Sugarcane yield  | Average yield in Brazil in 2014–2018: 73.8 ton/ha/year (range: 70.6–75.2 ton/ha/year)                                   |   |
| Emissions from crop residues   | Direct N <sub>2</sub> O emissions from crop residues: 0.8 kg N <sub>2</sub> O/ha  | Supplier information & N <sub>2</sub> O emissions calculated according to Agri-footprint v5 |

processed immediately.

In the sugar mill, sugarcane is pressed to extract the juice and a fibrous residue remains, known as bagasse. The sugar juice is used to make sugar and ethanol while bagasse is burned in the CHP (combined heat and power) plant. The juice is purified to remove the suspended matter and the resulting filter cake. The clarified juice is evaporated and crystallized until the required sugar purity. Molasses is obtained as a by-product from the crystallization steps. Both molasses and sugar are inputs to ethanol production. Ethanol is produced through yeast fermentation with the main by-product being vinasse [40].

Sugarcane is supplied by farms around the sugar mill, from a distance <50 km. The products of this sugar mill are sugar, hydrous and anhydrous ethanol, electricity, and steam. Other by-products are filter cake and vinasse, which are applied to the sugarcane fields as fertilizers. The steam and electricity generated from bagasse in the CHP plant is sufficient to run the mill and the excess electricity is sold to Corbion or to the Brazilian electricity grid. Corbion also purchases steam from the sugar mill, produced exclusively from bagasse. More information on the sugar mill by-products can be found in the [Multi-functionality section](#).

All inputs and outputs for the sugar mill, except the emissions to air, were based on supplier information (confidential). Emissions to air from bagasse combustion were included, based on the Agri-footprint V4 dataset “Sugar, from sugar cane, from sugar production, at plant/BR”.

### 3.2.1. Multi-functionality

The sugar mill is a multifunctional process, with the output products

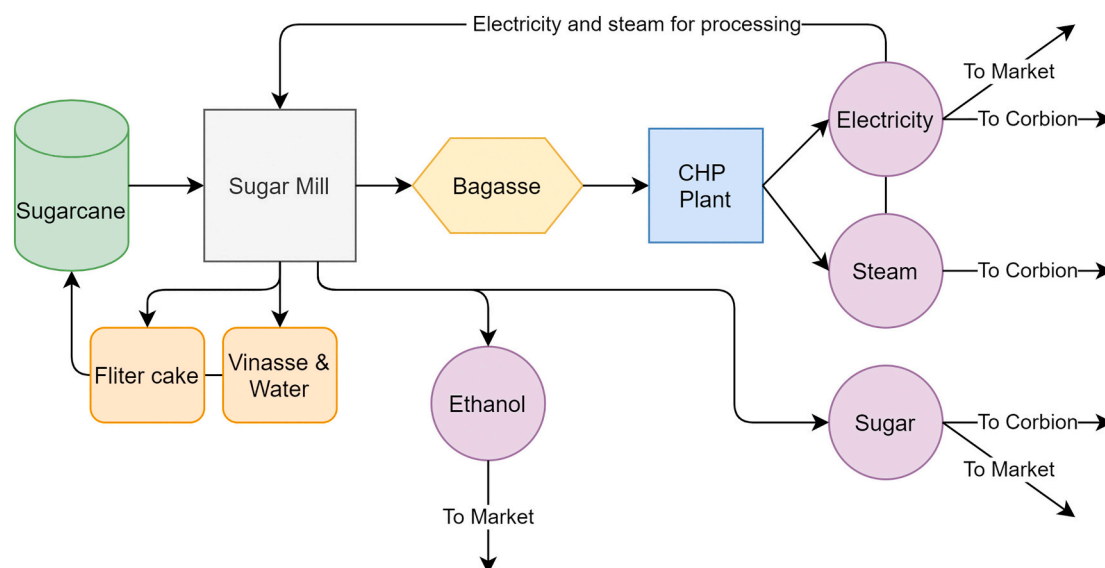


Fig. 2. Process scheme for the mill with inputs in yellow, products internally recycled in orange and outputs in purple. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

shown in Fig. 2: sugar, ethanol, electricity, steam, filter cake and vinsasse. From all these outputs, only sugar, ethanol, and electricity are sold directly to the market, including Corbion. Filter cake and vinsasse are returned to the sugarcane cultivation fields around the mill and the emissions from their application are included in the sugarcane dataset, as described in the previous paragraph. Additionally, it is assumed that the benefit of this practice in terms, for example, of improved yield is already reflected in the sugarcane dataset. Corbion also uses steam from the sugar mill because both sites are co-located. Steam is not sold to any other external parties and is not considered as a by-product in the data sources provided by the mill. If steam was not used by Corbion, it would either be wasted or used to generate additional electricity, with very limited benefit. For these reasons, no burdens are allocated to steam.

Therefore, relevant co-products of the sugar mill in terms of allocation are:

- Sugar
- Ethanol
- Electricity

As shown in Fig. 2, the production of these products is interlinked and cannot be sub-divided. In attributional modelling, when sub-division is not possible, allocation between co-products should be applied [41]. Economic allocation is applied as default based on the PEFCR for animal feed [23]. The economic allocation factors, based on average prices in 2016–2019, are 45%, 45% and 9% for sugar, ethanol and electricity, respectively. In the sensitivity analysis (see Section 4.3) energy allocation is applied, which results in slightly higher allocation factor for sugar and lower allocation factor for electricity, i.e. 48%, 45% and 7%.

### 3.3. Algae production and downstream processing

At the Corbion facility, algae of the genus *Schizochytrium* sp. are cultivated in large-scale stainless-steel agitated aerobic fermenters. *Schizochytrium* sp. belongs to the family, Thraustochytriaceae, and is a member of the Chromista kingdom. Historically, it has been grouped with algae (CEN definition EN 17399 2020) and for this reason the term algae is commonly used.

Sugar syrup is prepared and mixed with other nutrients, including nitrogen sources, to make up the fermentation medium for the algae

growth and oil production. Oil production occurs intracellularly. The main co-product of the fermentation is CO<sub>2</sub> which is released to the atmosphere.

The algae broth is harvested and dried using steam from bagasse. The dry algae biomass is a free-flowing dry powder with oil high in DHA encapsulated within the algae biomass. The water removed in the drying steps is condensed and reused in the process. Algae omega-3 DHA liquid suspension is produced by mixing algae biomass with vegetable oil. The liquid suspension format allows for several benefits including more efficient bulk transportation and, in some cases, easier and higher levels of incorporation into feed.

As described earlier, the sugar, electricity and steam consumed at the Corbion facility are generated from sugarcane and sourced from the nearby sugar mill. The wastewater effluent is also returned to the sugar mill where it is treated and applied to the cane plantations. Additional background data, for example for the fermentation nutrients and other auxiliary chemicals, is included using suitable Ecoinvent 3.6 APOS datasets. The production of vegetable oil is based on the Agri-footprint database, including DLUC based on crop and sourcing country [42]. For the default case, consistently with the sugar mill model, economic allocation is used.

The LCI for algae omega-3 DHA production is based on the confidential process and for this reason detailed input/output data cannot be disclosed.

## 4. Results

### 4.1. Life Cycle Impact Assessment results

The Life Cycle Impact Assessment (LCIA) results for the six most relevant impact categories recommended by the feed PEFCR are shown in Table 2. The impacts of algae omega-3 DHA powder and liquid suspension are quite similar. Algae omega-3 DHA liquid suspension has a slightly larger impact than algae omega-3 DHA powder for the climate change, acidification and eutrophication impact categories, and a slightly lower impact is observed for particulate matter, land use and water use categories. Fig. 3 shows the process contribution to each of these impact categories which is described in more detail in the next paragraphs.



**Table 2**

Results for the relevant impact assessment categories for 1 kg of omega-3 in algae omega-3 DHA (Docosahexaenoic acid) powder and liquid suspension, using Environmental footprint (EF) 3.0 method.

| Impact category             | Unit                    | Algae omega-3 DHA powder | Algae omega-3 DHA liquid suspension |
|-----------------------------|-------------------------|--------------------------|-------------------------------------|
| Climate change              | kg CO <sub>2</sub> eq   | 4.12                     | 4.70                                |
| Particulate matter          | Disease inc.            | 1.35E-06                 | 1.23E-06                            |
| Acidification               | mol H <sup>+</sup> eq   | 5.65E-02                 | 6.91E-02                            |
| Eutrophication, terrestrial | mol N eq                | 1.82E-01                 | 2.50E-01                            |
| Land use                    | Pt (soil index quality) | 2090                     | 1802                                |
| Water use                   | m <sup>3</sup> depriv.  | 0.926                    | 0.757                               |

## 4.2. Process contributions

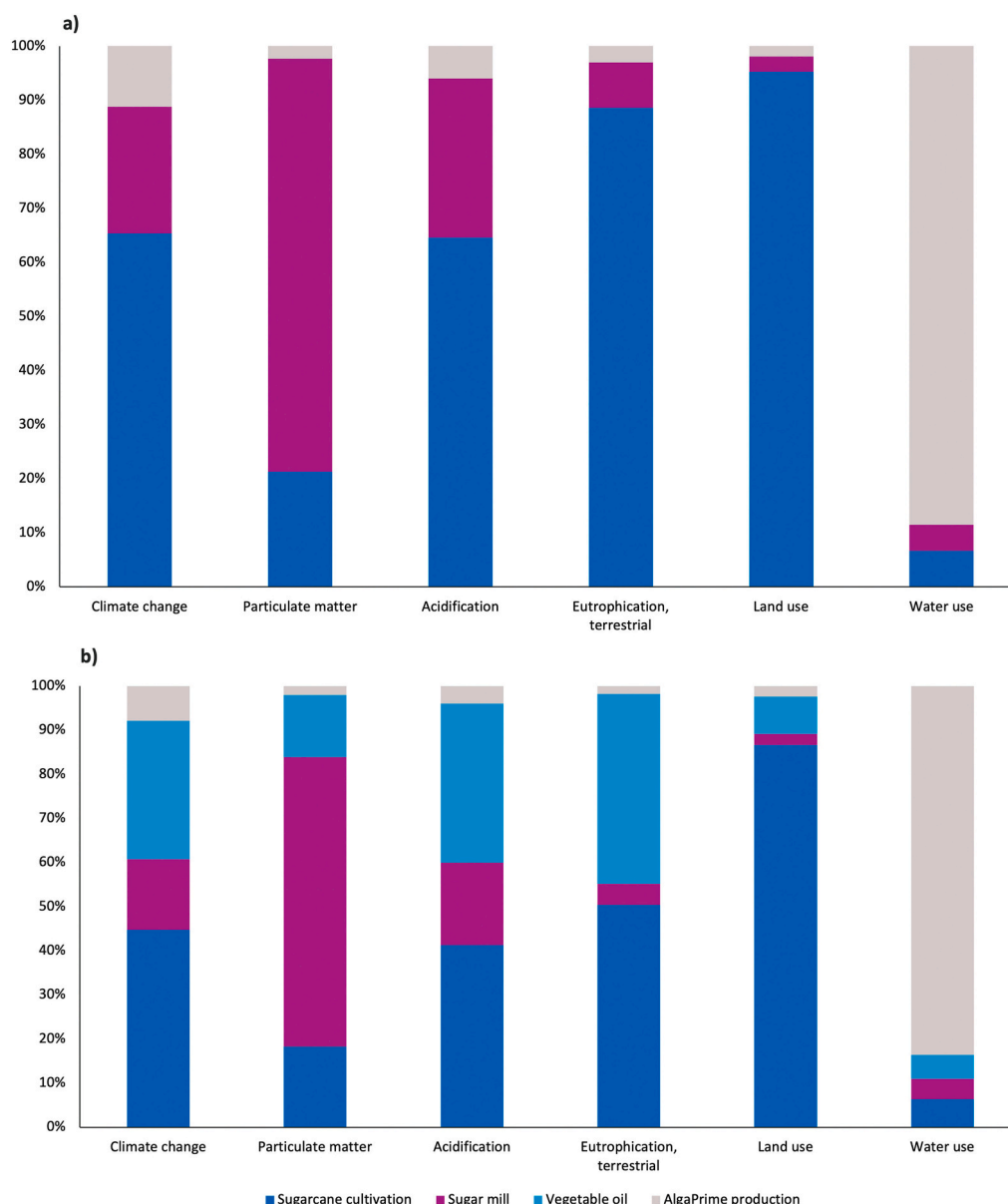
### 4.2.1. Climate change

The GHG emissions related to sugarcane cultivation contribute 65% to the total climate change impact of algae omega-3 DHA powder.

Sugarcane is the input used for both the feedstock and energy production for algae omega-3 DHA production. Most of these emissions (57%) were due to direct N<sub>2</sub>O and CO<sub>2</sub> emissions resulting from crop residues, synthetic fertilizer application and the application of the by-products, vinasse and filter cake. The remaining emissions are attributed to diesel use for agricultural machinery (20%), fertilizer production (13%), and production of crop protection products (5%). Based on the study conducted by GRAS, the CO<sub>2</sub> emissions related to direct land use change were determined to be 0.

The second largest contributor to the GHG emissions of algae omega-3 DHA powder is the sugar mill which represents 22% of the product emissions. These emissions are driven by the transport of the cane by truck to the mill (49%) and the N<sub>2</sub>O and CH<sub>4</sub> emissions from bagasse combustion (50%). The small amount of chemicals used in sugarcane processing represent the remainder of the GHG emissions in the sugar mill.

For algae omega-3 DHA powder production, some additional nutrients and chemicals are used which represent 11% of the total GHG emissions. Direct process emissions are mostly biogenic CO<sub>2</sub> formed in



**Fig. 3.** Contribution of different process stages for (a) algae omega-3 DHA (Docosahexaenoic acid) powder and (b) algae omega-3 DHA liquid suspension.

the fermentation, which is not considered for GWP (global warming potential).

Regarding algae omega-3 DHA liquid suspension, the production and transport of vegetable oil contributes 31% to the impact on GHG emissions. Cultivation of vegetable oil is the most relevant step, again driven by direct  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions from fertilizer application, fertilizer production, energy used for agricultural machinery and to a smaller extent to DLUC. According to FAO statistics, over the last 20 years, there was about 0.1% land transformation from perennial crop to the oil crop resulting in some GHG emissions from DLUC [42].

#### 4.2.2. Particulate matter

The impact category of particulate matter refers to the emission of fine particles and its precursors (e.g.,  $\text{NO}_x$  and  $\text{SO}_2$ ) and the adverse potential impact on human health.

Fig. 3 shows that most of the emissions related to algae omega-3 DHA powder come from the sugar mill (76%). Particulates and sulfur dioxide emissions from bagasse burning contribute the most for these emissions. Sugarcane cultivation is the second largest contributor (21%) with ammonia emissions from fertilizer having the highest influence. Likewise, for algae omega-3 DHA liquid suspension, ammonia emissions from fertilizer application in oil crop cultivation contribute to particulate matter.

#### 4.2.3. Acidification

Acidification is caused by the emission of the compounds  $\text{NH}_3$ ,  $\text{NO}_2$  and  $\text{SO}_x$  which are the precursors to acid rain. When these compounds are deposited to the soil or freshwater, the acidity can change. As most ecosystems operate at an optimal acidity level, changes in the acidity can have drastic repercussions on ecosystem functionality and individual species.

According to Fig. 3, the largest impact to acidification potential is the cultivation of the sugarcane. Emissions of ammonia and nitrous oxide due to the application of fertilizer, both synthetic and organic, are the largest contributors, representing 65% of the acidification impact of algae omega-3 DHA powder. The main reason for acidification at the sugar mill is the sulfur dioxide emitted from the combustion of the bagasse. The additional emissions that impact the acidification potential can be linked to transportation and production of fertilizers and chemicals used throughout the product system.

For algae omega-3 DHA liquid suspension additional impacts are related to vegetable oil crop production and emissions from fertilizer application.

#### 4.2.4. Eutrophication

Terrestrial eutrophication refers to the deposition of aerial nitrogen compounds such as  $\text{NO}_x$  and  $\text{NH}_3$  to terrestrial environments resulting in increased nutrient availability. As seen in Fig. 3, sugarcane cultivation accounts for 89% of the impacts in terrestrial eutrophication of algae omega-3 DHA powder production. These emissions are related to nitrogen and phosphate fertilizer application. Again, for algae omega-3 DHA liquid suspension, additional impacts are related to vegetable oil crop production and emissions from fertilizer application.

The remaining impacts contribution to eutrophication are associated with the emission of  $\text{NO}_x$  throughout the product system. Most of these remaining emissions are credited to the combustion of fuel during transportation.

#### 4.2.5. Land use and water use

Land use is an essential topic when discussing food systems and development of alternative feed ingredients. The land use impact of algae omega-3 DHA liquid suspension product is dominated by sugarcane cultivation (87%) and vegetable oil production (8%), see Fig. 3.

The water scarcity for each of the process steps can be seen in Fig. 3. The water use impact from sugarcane cultivation is not significant ~6–7% of total water use impact because sugarcane is not an irrigated

crop and in the region of Orindiúva, the water stress level is low (see Fig. 4). More than 80% of the water use impact is contributed to the water used in the algae production. The water consumed at the algae production facility is withdrawn from the local river and in the same region as the sugarcane plantations, therefore from a low water stress level. The water consumption is mainly due to the evaporation in the cooling towers. Water used in the fermentation is largely recycled within the process and a smaller amount is returned to the sugar mill where it is treated and applied in the sugarcane fields.

#### 4.3. Sensitivity analysis

Sensitivity analyses are performed to estimate the effect of the choices made regarding methods and data assumptions on the outcome of a study. The variables considered in the sensitivity analysis were determined based on key uncertain assumptions and on the relevant contributions to the LCIA results. The variables include key aspects related to algae production, the sugar mill, and sugarcane cultivation:

- 1) Sugar conversion yield to omega-3 DHA
- 2) Allocation of by-products using energy allocation factor
- 3) Amount of electricity exported from the sugar mill
- 4) Sugarcane yield

The algae production data has low uncertainty because of the high maturity level of the process and the accuracy of the engineering models. Sugar conversion yield to omega-3 DHA is included in the sensitivity analysis since it can potentially be further improved as an outcome of the R&D efforts on *Schizochytrium* strain development. Considering an increase of 5–20% in the conversion yield of sugar to omega-3 DHA, a corresponding 5–20% decrease in the environmental footprint of algae omega-3 DHA is expected. The yield increase reduces the environmental impact for all categories because the sensitivity analysis assumes that all other process parameters remain unchanged.

The choice of the allocation method is an important assumption in any LCA and the ISO 14044 requires that a sensitivity analysis is conducted to illustrate the consequences of the selected approach [18]. The default results in this study are based on economic allocation of the by-products and, as part of the sensitivity analysis, the results were also calculated using energy allocation. This alternative calculation provides insights on the impact of the modelling choice for allocation and showed that energy allocation results in lower environmental impacts. The difference between both allocation approaches is less than 3% for algae omega-3 DHA powder and less than 9% for algae omega-3 DHA liquid suspension, for all impact categories investigated. Algae omega-3 DHA liquid suspension is slightly more sensitive to the allocation approach because of the vegetable oil.

The amount of electricity exported by the sugar mill is a potential measure to reduce the environmental impact of the sugar mill products. This can be achieved by increasing the energy efficiency of the sugar and ethanol production, by implementing energy intensification measures or by improving the efficiency of the CHP. In the sensitivity analysis, higher values of electricity export were considered, up to 120 kWh/ton sugarcane (about 200% larger than the default case). The higher value of electricity export considered is achieved for modern mills with high pressure co-generation [43]. For all impact categories, the emissions decrease with the amount of electricity exported by the mill. The effect of increasing the electricity export is the largest for land use and particulate matter and almost negligible for water (See Fig. 5). Even if a maximum electricity export assumed, the GHG emissions associated with algae omega-3 DHA liquid suspension decreases by less than 7%.

Crop yield can have a significant impact on the overall emissions from crops and is highly dependent on the farming practices and weather conditions, such as rainfall. As mentioned in Section 3.1, the sugarcane yield was based on the most recent 4-year national average. Even though this average is aligned with the information provided by

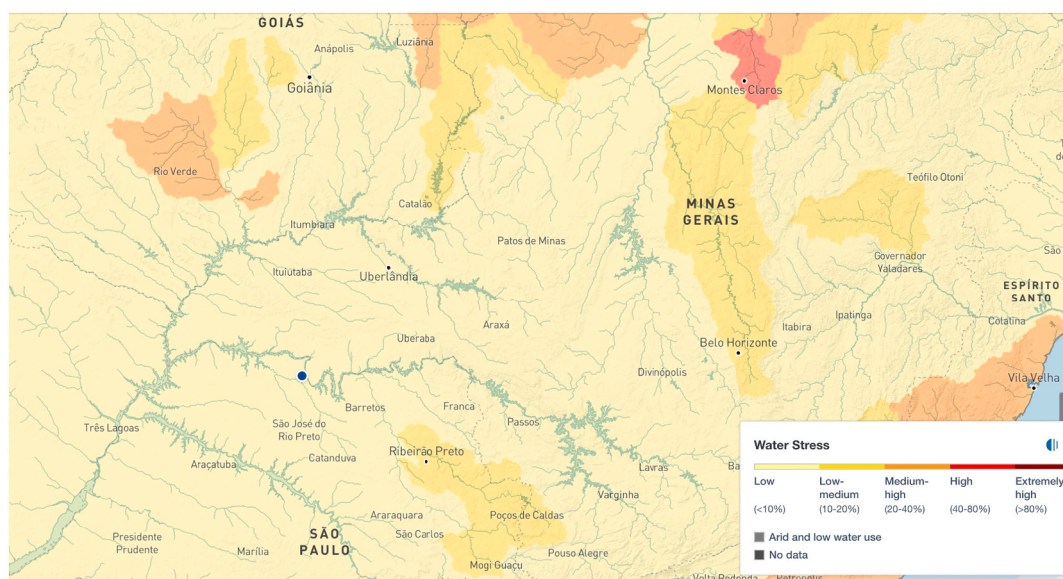


Fig. 4. Water stress map of the area of Orindiúva (World Resource Institute, WRI – [29]).

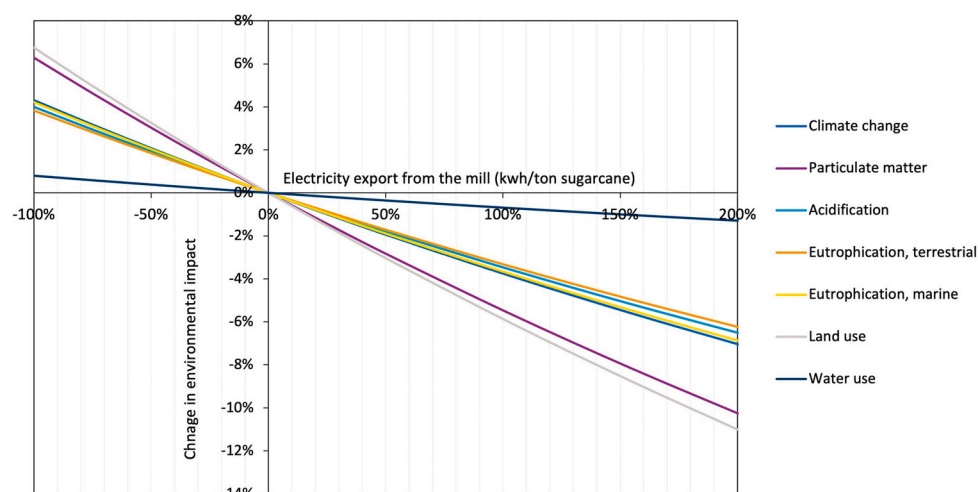


Fig. 5. Effect of electricity export on the environmental impact of algae omega-3 DHA (Docosahexaenoic acid) liquid suspension. x-axis: percent change in the input parameter electricity export (kwh/ton sugarcane) and y-axis: relative change in the environmental impact. The 0% change for electricity export corresponds to the default case.

the supplier for 2019, it was also indicated that the yields varied within the plantation areas. Fig. 6 shows the change in the impacts of algae omega-3 DHA liquid suspension resulting from the variation of the sugarcane yield. The range of the sugarcane yield considered (60–90 ton/ha/year, baseline yield 73.8 ton/ha/year) covers the variation in the last 5 years (Table 1) and the expected yield improvement in the next 5–10 years. A zero change in input corresponds to the default case, a positive change in input corresponds to an increase in yield. The yield increase has a positive impact in reducing all environmental categories. Impact categories that are most sensitive to sugarcane yield are: land use, eutrophication (marine and terrestrial) and climate change.

From the sensitivity analysis, it is concluded that the sugarcane yield and the conversion yield of sugar to omega-3 DHA are the most sensitive parameters. The allocation choice and amount of electricity export have a lower impact on the results.

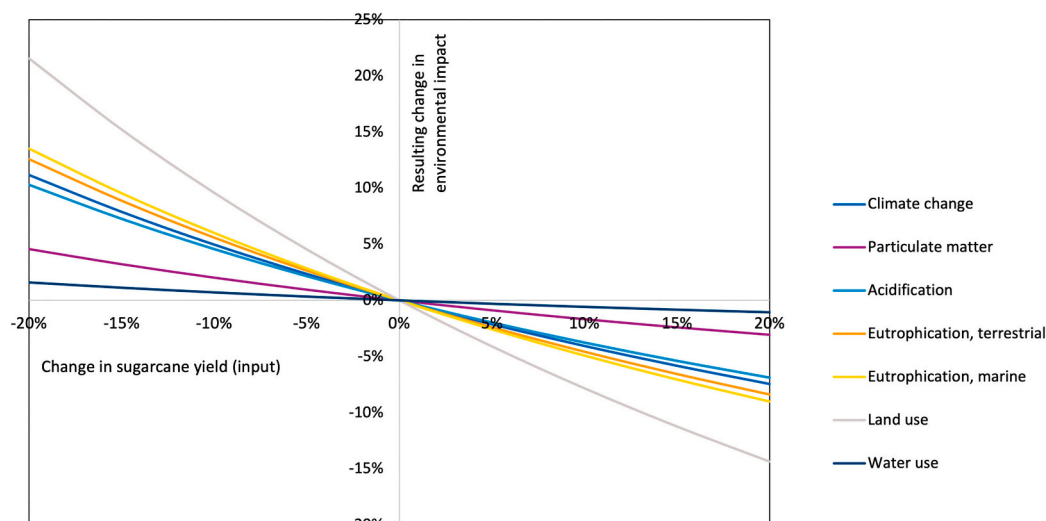
## 5. Discussion

### 5.1. Comparison with fish oil omega-3

The production of fish oil relies on the capture of small pelagic marine fish and fish trimmings from fish processing facilities. The wild fish are caught and transported to fishmeal plants and then processed into fish meal and fish oil (FMFO).

The comparison with fish oil was based on data available in the databases Agri-footprint v5 economic (arithmetic average of the datasets available for the seven different countries “Fish oil, at processing/countries Economic”, countries = Denmark, Chile, Peru, Norway, Germany, Great Britain, The Netherlands) and Ecoinvent 3.6 (Fish oil, from anchovy {GLO} | market for fish oil | APOS). The Agri-footprint datasets were modified to reflect the average market prices of fish oil and fish meal in the period of 2015–2019 [44]. Based on these prices, the allocation factors are 22% and 78% for fish oil and fish meal, respectively. For the Ecoinvent economic allocation, the original factors were used: 31% and 69% for fish oil and fish meal, respectively. For the fish oil





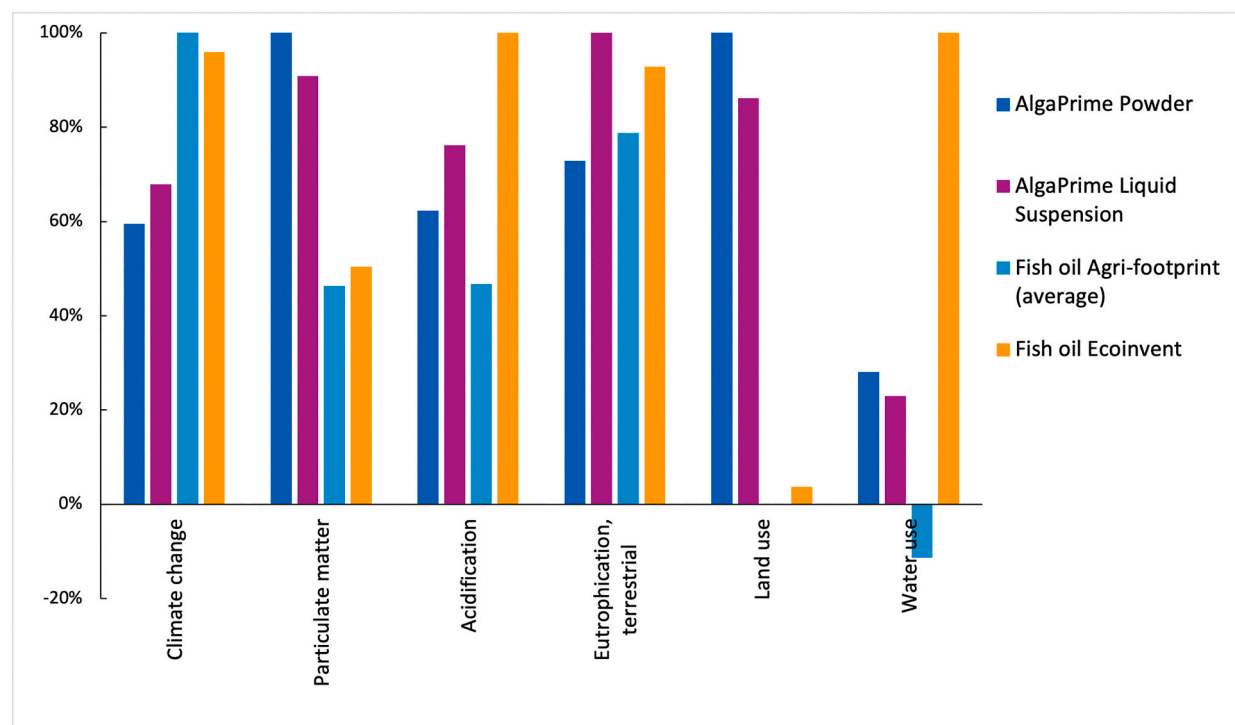
**Fig. 6.** Effect of sugarcane yield on the environmental impact of algae omega-3 DHA (Docosahexaenoic acid) liquid suspension. x-axis: percent change in the input parameter sugarcane yield and y-axis: relative change in the environmental impact. The average slope of the curves is the relative sensitivity parameter.

system, these datasets consider the entire production process, including capture of wild fish, use of offal and processing of fish into fish meal and fish oil.

The omega-3 content of fish oil varies based on species of fish, time of year, diet, and many other factors. Fish oil sourced from the North Atlantic generally has a lower omega-3 content (maximum 16%) while fish oil from anchovies in the South Pacific can be much higher (up to 24%). The omega-3 content of fish oil used for comparison was 19%, this represents the higher end of the spectrum of omega-3 content of fish oil that is being used in aquafeed and is representative of the global average.

The reference flow for the comparison of fish oil with algae omega-3

DHA is 1 kg of omega-3 fatty acids, assuming 30% of long chain omega-3 fatty acids for the algae omega-3 DHA products and 19% of long chain omega-3 fatty acid for fish oil. Fig. 7 shows that algae omega-3 DHA has lower impact than fish oil in the climate change impact category. The impacts of fish oil production are mostly related to the diesel energy consumption for the fishing vessels (diesel engine and generating of power for cooling), and the electricity and heat to operate the FMFO plant. Both the type and efficiency of the fishing vessel, and the efficiency and source of energy used at the FMFO plant, have a significant influence on the results for fish oil. The most in depth LCA study of fishmeal plants to date highlighted the processing phase as the most impactful stage of fish oil production [45].



**Fig. 7.** Results for the environmental footprint of algae omega-3 DHA (Docosahexaenoic acid) and omega-3 from fish oil. The fish oil datasets are based on Ecoinvent 3.6 “Fish oil, from anchovy {GLO} | market for fish oil | APOS” and the average of the Agri-footprint v5 datasets (Chile, Perú, Norway, Denmark, UK, Netherlands and Germany) The different products are compared per 1 kg of omega-3s and the maximum value is set to 100%.

The algae omega-3 DHA products have relatively higher impact on particulate matter than fish oils, which is related with the energy production. Land use for fish oil omega-3 is low – instead of agricultural land, the fisheries use marine resources which are not defined by the LANCA model. Regarding water use, the results from fish oil differ substantially between both databases and conclusions cannot be drawn. Water consumption in the Ecoinvent dataset is related to water use in the FMFO plant. For all other impact categories, results are driven by the significant fossil fuel consumption to produce fish oil and diesel for the fish capture.

### 5.2. Comparison with other algae systems

The LCAs available in the literature from heterotrophic algae are sparse and focus on biodiesel [13–15] and food or feed ingredient production [16]. These studies are based on lab, pilot or literature data, and include additional processing steps (e.g., oil extraction, biodiesel production, lyophilization, etc.) which are not required for algae omega-3 DHA production and have different functional units. For these reasons, the results cannot directly be compared.

Studies on biodiesel production show that the GWP of the heterotrophic and autotrophic production systems are within the same range, depending on the scenarios evaluated [14,15]. In terms of land use, the autotrophic systems have a better performance, and for water, the results depend on the feedstock. [14]. The study of Smetana et al. (2017) concluded that the heterotrophic production system results in the lowest environmental impact. The use of renewable energy for algae omega-3 DHA production is one of the main factors for achieving a low environmental footprint [16]. For the heterotrophic systems, the choice of feedstock and sourcing geography is very impactful [13] and substantiated in this study by the high contribution of the sugarcane cultivation to the LCA.

### 5.3. Limitations of the study and recommendations

Heterotrophic omega-3 DHA microalgae have been produced at industrial scale for several years at Corbion. The algae production facility also produces other algae products currently. Measured plant data is not directly used for the LCI because it is not representative of the algae facility producing solely omega-3 DHA at full capacity. For this reason, the LCI was based on calculated values based on heat and mass balances combining both historical data and process design knowledge. It is recommended that the LCA is updated with measured plant data for the products when this is available and representative. However, no substantial change is expected because the impact of algae omega-3 DHA manufacturing was shown to be low in most impact categories. The LCA results are representative only for heterotrophic algae production using this commercial process and integrated with the sugar mill, both for feedstock and energy supply.

Although this study followed the guidelines of the PEFCR measurement for the most relevant impact categories, the study does not quantify all environmental impacts. For example, the depletion of marine resources is not included in any LCA impact categories and the impact caused by unsustainable fisheries cannot be immediately assessed.

Additionally, one of the most important impact categories to be considered in the future will be biodiversity loss. Agriculture and fishing are major contributors to biodiversity loss and should be considered when discussing impacts of these industries [46]. Measuring biodiversity and properly defining its worth has been a challenge and even more challenging when trying to compare between terrestrial and marine aquatic environments. There are currently methodologies in development that may make this possible in the near future and should be considered when making decisions about the environmental impact of both of these systems [6,47,48].

## 6. Conclusions

With the global supply of forage fish at a plateau, there is growing pressure to reduce dependence on the use of fish oil in aquaculture and livestock feeds, as well as pet food. The use of algae omega-3s as a scalable and alternative to fish oil is growing. This LCA provides a detailed description of the environmental impacts of two commercial omega-3 DHA products produced from heterotrophically grown microalgae.

For both products, algae omega-3 DHA powder and liquid suspension, sugarcane cultivation has the largest contribution for most of the categories, except for particulate matter and water use. The last two impacts are, respectively, dominated by the sugar mill operation and algae production. The sensitivity analysis showed that the environmental impacts are highly sensitive to sugarcane yield. In contrast, the allocation methods and amount of electricity export as a credit, had a limited influence on the results.

The comparison with fish oil was carried out using data available in LCI databases. The large range of results obtained for the different datasets of fish oil, and the variability of the omega-3 content of fish oil, made the comparison challenging. Despite the ranges observed in the impacts of fish oil from different LCI databases, both algae omega-3 DHA products offer lower impacts for climate change and acidification compared to fish oil. On the other hand, the particulate matter impact is higher for algae omega-3 DHA products due to the air emissions from bagasse combustion. In addition, the land use impact for fish oil appears to be lower – because marine fisheries are not defined by the current LCA land use impact model.

In conclusion, the use of algae omega-3 DHA in feed can contribute positively to maintaining or improving omega-3 levels in feed, reduce pressure on marine resources, and play a role in improving the carbon footprint of feed formulations.

Looking forward, the production of heterotrophic algae omega-3 DHA at commercial scale still has optimization potential, for instance through the development of higher sugar to omega-3 DHA yield algae strains and intensification of the production process to increase energy and water efficiencies. Additionally, reductions can be achieved through engagement with the sugar mill on potential improvements both for sugarcane cultivation and the sugar mill. Likewise, sourcing low impact vegetable oil can improve the footprint of the algae omega-3 DHA liquid suspension format.

### CRedit authorship contribution statement

All authors contributed equally to the conception of the manuscript and to its drafting, and approval. Dillon North-Davis and Ana Morão were the main contributors to the acquisition, analysis and interpretation of the data.

### Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

### Declaration of competing interest

Ana Morao reports a relationship with Corbion BV that includes: employment. Jill Kauffman Johnson reports a relationship with Corbion CV that includes: consulting or advisory.

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## Appendix A. Supplementary data

The LCI datasets can only be accessed with SimaPro 9.1. Importing the data into other LCA softwares could lead to errors due to incompatible elementary flows. Before any reuses of the dataset, please carefully check the goal and scope definition, the impact categories covered, and the choices made in the inventory modelling. The information can be found in Chapter 2 of the article. These LCI datasets do not offer information beyond the defined scope of the study. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2021.102494>.

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