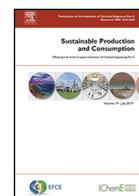




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## Research article

## Life cycle assessment of fish oil substitute produced by microalgae using food waste

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## ABSTRACT

Fish oil has been used in conventional aquaculture for decades, despite the known links between increasing global demand for fish and depletion of natural resources and vital ecosystems (FAO, 2020, 2019). Alternative feed ingredients, including algae oil rich in *docosahexaenoic acid* (DHA), has therefore been increasingly used to substitute traditional fish oil. Heterotrophic algae cultivation in bioreactors can be supported by a primary carbon feedstock recovered from food waste, a solution that could reduce environmental impacts and support the transition towards circular food systems. This study used life cycle assessment to quantify environmental impact of DHA produced by the heterotrophic algae *Cryptocodinium cohnii*, using short-chain carboxylic acids derived from dark fermentation of food waste. The future potential of DHA from algae was evaluated by comparing the environmental impact to that of DHA from Peruvian anchovy oil. With respect to global warming, terrestrial acidification, freshwater eutrophication and land use, algae oil inferred -52 ton CO<sub>2</sub>eq, 3.5 ton SO<sub>2</sub>eq, -94 kg Peq, 2700 m<sup>2</sup> eq, respectively per ton DHA. In comparison, the impact per ton DHA from fish oil was -15 ton CO<sub>2</sub>eq, 3.9 ton SO<sub>2</sub>eq, -97 kg Peq and 3200 m<sup>2</sup> eq. Furthermore, algae oil showed lower climate impact compared to canola and linseed oil. By including Ecosystem damage as indicator for ecosystem quality at endpoint level, the important aspect of biodiversity impact was accounted for. Although the method primarily accounts for indirect effects on biodiversity, DHA from algae oil showed lower Ecosystem damage compared to fish oil even when future energy development, optimized production, increased energy demand and effects on biotic resources were considered via sensitivity analyses. As the results suggest, algae oil holds a promising potential for increased sustainability within aquaculture, provided that continued development and optimization of this emerging technology is enabled through active decision-making and purposeful investments.

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## 1. Introduction

Today, aquaculture is the fastest growing food-producing sector, originally developed to support an increasing global population with nutritious food and essential Omega-3 fatty acids (FAO, 2018). Fish oil has been one of the most important ingredients in conventionally produced aquaculture feed for decades, primarily since it contains bioavailable polyunsaturated fatty acids, including the 6-fold unsaturated fatty acid *docosahexaenoic acid* (DHA). The original aim in farming high-value fish was to preserve marine biodiversity while supporting an increased global demand for food fish (European Commission, 2019). However, current fish oil supply is

highly dependent on fossil energy and marine raw materials, and research has shown that natural resources and ecosystems are being depleted as the global demand for fish increases (FAO, 2020, 2019).

In aquatic ecosystems, DHA is naturally produced by planktonic microalgae and is accumulated in fish via the food web (Colombo et al., 2020; Sprague et al., 2017). As DHA cannot be synthesised by animals, a sufficient intake must be obtained through the diet. Therefore, fish oil rich in DHA is often added in food and feed production to enhance nutrition levels in dairy, meat and fish consumed by humans (Silva et al., 2018; Toppe, 2013). Each year, around 1 million tons of fish oil is used to produce aquafeed (Beal et al., 2018), where Peruvian anchovy oil contains one of the highest concentrations of DHA (IFFO, 2017). Conventional fish oil production depends on marine raw materials, primarily for-

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age fish such as anchovy, and finite resources such as fossil energy to power the conversion processes (Rodríguez et al., 2019; Tyedmers et al., 2006). Fish oil production has also been shown to contribute to two of the most urgent threats to life on Earth: global warming and loss of biodiversity (Center for Biological Diversity, 2020; Ghamkhar and Hicks, 2020; United Nations, 2019). Moreover, due to global warming the natural DHA synthesis by marine microalgae is predicted to decrease by 58% until 2100 (Colombo et al., 2020), which would reduce the natural DHA content in fish oil. In addition, long-term monitoring of marine fish stocks by FAO (2020) showed that 34% of fish stocks were overfished in 2017. Thus, the dependency on fish oil for aquafeed must be reduced to ensure future food security (Cottrell et al., 2020) and to achieve sustainable market growth of aquaculture (Hardy, 2010).

New production methods with lower environmental impact are needed to maintain vital ecosystems, increase resource efficiency and support future sustainability within food supply chains. A potential solution to the impact associated with conventional fish oil production could be to gain DHA directly from the marine primary producers, namely microalgae. Using microalgae as a novel aquafeed could decrease the demand for forage fish in aquaculture, while still maintaining the required DHA profile for high-value aquaculture fish feed (Beal et al., 2018; Sprague et al., 2017, 2015). Previous research has established that heterotrophic microalgae can be cultivated in bioreactors using carbon sources derived from food waste as primary feedstock (Chalima et al., 2020, 2019, 2017), thus enabling an alternative resource recovery solution within food waste management.

Today, about 1.3 billion ton food is wasted globally each year, of which over 900 million ton origin from households, retail establishments and the food service sector (UNEP, 2021). Food waste accounts for a considerable proportion of environmental burden for the current food supply chain, especially since current practice are often based on a linear production process (Laso et al., 2018). Increased resource recovery and nutrient recycling are considered key actions to achieve long-term sustainability, especially within the future food system and food waste management. Life cycle assessment (LCA) is a method commonly used to systematically assess and quantify the environmental impact for a process or product. By considering resource use and emissions related to the whole production chain, LCA is a valuable tool for evaluating if a suggested solution can reduce the environmental impact compared with a reference scenario. The aim of this study was to evaluate the future potential of DHA produced from algae with substrate originating from DF using food waste, by assessing and comparing the environmental impact to that of DHA produced from Peruvian anchovy oil. A quantitative assessment of the environmental impact and the potential effects on biodiversity was included to provide a vital dimension of aquaculture and food waste valorisation to policymakers, the research community and the industry. The long-term goal with this study was to support sustainable development, by assessing innovative solutions for food waste valorisation within a circular economy approach.

## 2. Literature review

The environmental impact of conventional fish oil production has been thoroughly studied by previous research (Avadí and Fréon, 2013; Fréon et al., 2017; Silva et al., 2018). To increase sustainability within aquaculture and reduce dependency on traditionally used marine raw material, alternative DHA sources in aquafeed has gained a lot of scientific attention (Bélangier-Lamonde et al., 2018; Glencross et al., 2020). Vegetable sources, such as canola or linseed oil, require conversion of *alpha lipoic acid* (ALA) via digestion to provide bioavailable DHA (Kannan et al., 2021; Russo et al., 2021). Although often considered controversial,

genetically modified crops has been shown to increase DHA potential in plants to levels comparable with fish oil (Petrie et al., 2020; West et al., 2021; Zhou et al., 2019). An alternative source that provide bioavailable DHA are yeast oil and algae oil (Parsons et al., 2019; Porcelli et al., 2020; Yarnold et al., 2019). Research suggest that microbial oils from heterotrophic algae and yeast will likely be common within future food systems (Parsons et al., 2018), but microalgae oils are considered one of the most promising future fish oil substitutes (Cottrell et al., 2020; Oliver et al., 2020). Both total and partial replacement of fish oil in aquafeed is currently possible, but the nutritional composition of the substituting oil must be considered to maintain the required nutritional value in food fish (Sarker et al., 2016; Schade et al., 2020; Sissener, 2018). Ghamkhar and Hicks (2020) concluded that sole fish oil replacement can infer reduced stress on biotic resources, but they also highlighted that technologies for producing substituting oils needs further improvement to achieve more effective energy use and mitigate potential burden shifts. Commercial algae derived products, including DHA for feed and food, are well established on the market and will likely be further developed to improve effectiveness and reduce production costs (Patel et al., 2020a; Yarnold et al., 2019).

*Thraustochytriaceae* and the dinoflagellate *Cryptecodinium* sp. are eukaryotic, planktonic heterotrophic marine microalgae that are known to accumulate DHA to beyond 10% (w/w) of their cell weight (Kumar et al., 2021; Mendes et al., 2009). The intensively examined strain *Cryptecodinium cohnii* (*C. cohnii*) is cultivated aerobically in bioreactors supported by a carbon source as primary feedstock. Different feedstock has been frequently used, including glucose (Deprá et al., 2020), lignocellulosic biomass (Karnaouri et al., 2020) and olive pomace (Paz et al., 2020). Acetic acids and other short-chain fatty acids has been shown to greatly benefit microalgae DHA accumulation (Hillig, 2014; Sijtsma et al., 2010), especially waste derived *volatile fatty acids* (Oliver et al., 2020). Volatile fatty acids (VFA) are obtainable from anaerobic digestion (AD), in particular dark fermentation (DF) (Chalima et al., 2019; Fei et al., 2015; Patel et al., 2020a). DF is a common food waste (FW) valorisation process where the first two steps, hydrolysis and acidogenesis of the typical AD, yield short-chain carboxylic acids (mostly VFAs). VFAs extracted from AD or DF primarily consist of acetic acid (C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>) and smaller amounts of propionic acid (C<sub>3</sub>H<sub>6</sub>O<sub>2</sub>) and butyric acid (C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>), while the remaining biomass can still be used to produce biogas (Kim et al., 2019; Tampio et al., 2019). Previous studies by Paritosh et al. (2017) and Wainaina et al. (2019) suggest that up to 20 g VFA/L FW can be produced in the hydrolytic/ acidogenesis stage of conventional two-step AD, while up to 25 g VFA/L FW was suggested by Herrero Garcia et al. (2018) during optimised DF. Multiple studies have emphasised the prosperous potential for using VFA recovered from fermented FW as a sustainable alternative to conventional carbon sources for bioproduction of DHA from microalgae. The synthesis of valuable by-products like squalene can increase the worth of heterotrophic algae biomass (Patel et al., 2020b). Chalima et al., (2017) reviewed how VFA could be reused in a microalgae fermentation process, and a later study also included the VFA separation from DF and their use for the bioproduction to high added-value DHA by *C. cohnii* (Chalima et al., 2020, 2019). Patel et al. (2021) and Fei et al. (2015) both studied the effects on fatty acid accumulation when using VFA as primary carbon feedstock in heterotrophic algae cultivation. They found that a similar fatty acid accumulation could be obtained with VFA as when using traditional glucose, but the effect was largely affected by the amount and ratio of VFAs. Combining bioconversion of VFA to DHA through microalgae in combination with energy generation from FW, can infer increased sustainability within aquaculture (Oliver et al., 2020) and energy production (Chalima et al.,

2019; Paritosh et al., 2017). This approach also enables a novel solution for FW resource recovery, which has been widely recognised as an important component in sustainable development within the food system (Brancoli et al., 2020; Scherhauser et al., 2020; Teigiserova et al., 2020). As suggested by Woodhouse et al. (2018), primary production (including aquaculture and fishing) are generally main environmental hotspots in the food supply chain. Using a more circular flow of resources might reduce the burden from primary production, especially when considering fish oil substitutes. Current research suggest that microalgae will likely play an important role in maintained global food security by bridging a future gap between supply and demand for DHA (Jovanovic et al., 2021; Russo et al., 2021; Tocher et al., 2019). To ensure global food security and maintain nature's ability to provide resources in the future, substantial efforts are required to develop new production methods with lower environmental impact that also comply with the global Agenda 2030 and sustainable development goals (SDG) (Herrero et al., 2020; United Nations, 2020, 2019).

Identifying and understanding the environmental implications of new technologies is thus crucial to ensure that suggested new solutions also support future sustainability. Multiple studies have assessed the environmental impact of algae oil intended for the food supply chain (Beal et al., 2018; Porcelli et al., 2020; Schade et al., 2020). To date, previous LCA studies have mostly focused on phototrophic algae processes (Barr and Landis, 2018; Keller et al., 2017), where required inputs and outputs differ substantially from those in heterotrophic cultivation (Smetana et al., 2017). Similarly, assessments of algae oil used for biofuel production is more common than algae oil intended for food or feed (Hosseinzadeh-Bandbafha et al., 2020; Shi et al., 2019). However, Rösch et al. (2019) suggests that the inputs required for algae cultivation and harvest are often similar regardless of how the end product is used. In a recent study, Deprá et al. (2020) assessed environmental impacts with respect to commercial microalgae-based products, including DHA produced from *C. cohnii*. They concluded that microalgae as a source for DHA owns a high sustainable potential. Although commercialized in recent years, to our knowledge, no LCA study has yet assessed environmental impact of large-scale heterotrophic algae oil production intended to substitute fish oil.

Some of the most frequently included impact categories for seafood LCA are global warming, acidification and eutrophication (Ruiz-Salmón et al., 2021). Scherer et al. (2020) further suggest to also include land and sea use impact when addressing food security and biodiversity conservation, an aspect that alongside removal of fish stocks also has been highlighted by Langlois et al. (2015) and Hélias et al. (2018). Climate change, nutrient pollution, change in habitat, overexploitation and invasive species are so-called drivers of biodiversity loss that provide a measurable link between human actions and ecosystem damage (Watson et al., 2005). Even though frequently identified as especially important for LCA with a marine food focus, few previous studies have accounted for the impacts related to biotic resource use due to its complexity (Marques et al., 2021; Scherer et al., 2020; Winter et al., 2017). Following the cause-effect-chain in LCA, impact at midpoint infer damage to an area of protection at endpoint level. Impact at midpoint level causing loss of biodiversity infer damage to ecosystem quality, which in turn lead to Ecosystem damage. Biotic impact indicators are still under development and are therefore currently not included in commercial LCA methods (Crenna et al., 2020; Marques et al., 2021; Ruiz-Salmón et al., 2021). As suggested by Asselin et al. (2020), standardised LCA methods currently only cover three of the five identified drivers for biodiversity loss, while overexploitation and invasive species still need to be further developed. However, climate change, pollution and change in habitat at midpoint level can all be linked

to ecosystem quality and expressed in a common endpoint unit (Crenna et al., 2020; Huijbregts et al., 2016; Woods et al., 2016). Moreover, there is an urgent need improved *life cycle inventory* (LCI) data to fully evaluate the sustainability of microalgae cultivation (Avadí et al., 2020; Lopes da Silva et al., 2019).

### 3. Material and method

#### 3.1. Goal and scope

The LCA method (ISO, 2006a, 2006b) was used to assess environmental impact of fish oil substitute produced by microalgae using VFA derived from food waste as primary feedstock. By using an attributional (ALCA) approach, the aim was to identify resource-demanding flows and provide results via *life cycle impact assessment* (LCIA) that could support innovations within future DHA production and food waste management. The technology for large scale algae oil production using VFA from dark fermentation of food waste is currently in the development phase. To assess the system, laboratory and full-scale input and output data from previous studies was compiled and used to model a large scale DHA production. A physical functional unit (1 ton DHA) was selected to also reflect the nutritional function of the product, as proposed by McAuliffe et al. (2020). Data from Ecoinvent 3.5 were used for the background system (see Table A.1 in Appendix A), while substitution via system expansion was used in the foreground system to allocate the environmental burden between the two main by-products electricity and heat. System expansion was favoured to avoid economic or mass allocation, as suggested by the ISO 14040-series (ISO, 2006a).

#### 3.2. Description of scenarios

The studied systems were modelled as two parallel scenarios to assess large-scale production of DHA: a conceptual *Algae scenario*, where DHA was produced from *C. cohnii* microalgae using VFA from DF with food waste, and a conventional *Fish scenario*, where DHA was derived from Peruvian anchovy (Fig. 1). Included in the system boundary were production and end-of-life for required inputs, as well as construction of buildings and energy used for processing. Construction and maintenance of additional infrastructure were outside the scope of this study. Transport was included for inputs and outputs, while intermediate transport at the production site was excluded. NTMCalc Basic 4.0 was used to estimate transport distances for inputs, where a freight lorry of Euro class VI was assumed for most road transport.

The site location for algae oil and energy production was assumed to be Berlin, Germany, while fish oil production was assumed to be located in Lima, Peru. Site-specific data were primarily used, so the results are site dependent. The available food waste was considered a free resource and thereby did not contribute to the environmental impact, since production belongs to the preceding food system. The digestate was assumed to have a negligible market value in comparison with biogas and biohydrogen, therefore only transport from the AD plant was included, and not the end-of-life for digestate. Since the energy source strongly affects LCA results, a customised dataset for electricity and heat input and output was created using data from 2020 for the German electricity and heat production mix (see Table B.1 in Appendix B). Heat produced in Energy<sub>DF</sub> and Energy<sub>AD</sub> was assumed to be re-used during algae oil production, DF and AD. Avoided electricity due to biogas production was assumed to replace equal parts of the fossil energy sources lignite, natural gas and coal, while residual avoided heat was assumed to replace natural gas.

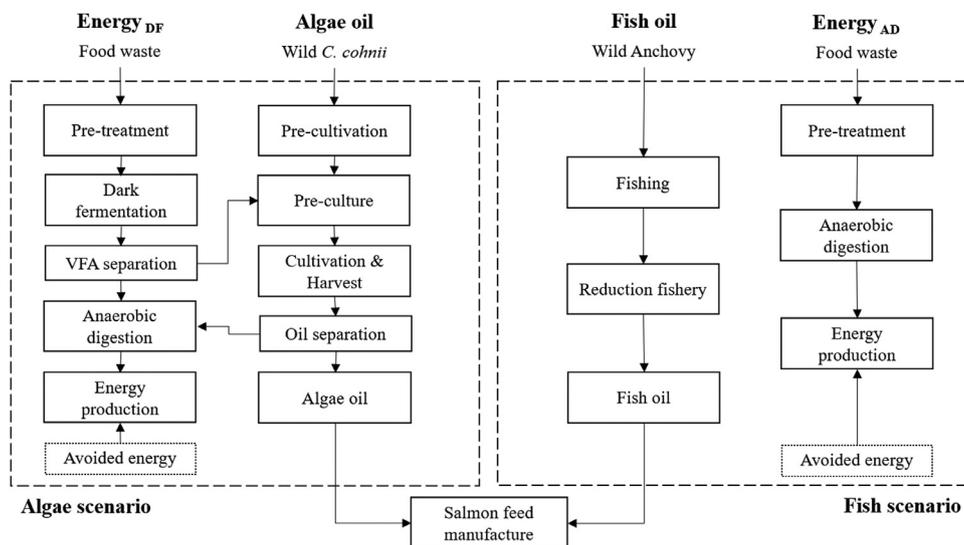


Fig. 1. Illustration of the model set-up for (left) the *Algae scenario* and (right) the *Fish scenario*. The dashed line represents the system boundary and the dotted line illustrates by-products included via system expansion. AD: anaerobic digestion, DF: dark fermentation, VFA: volatile fatty acids.

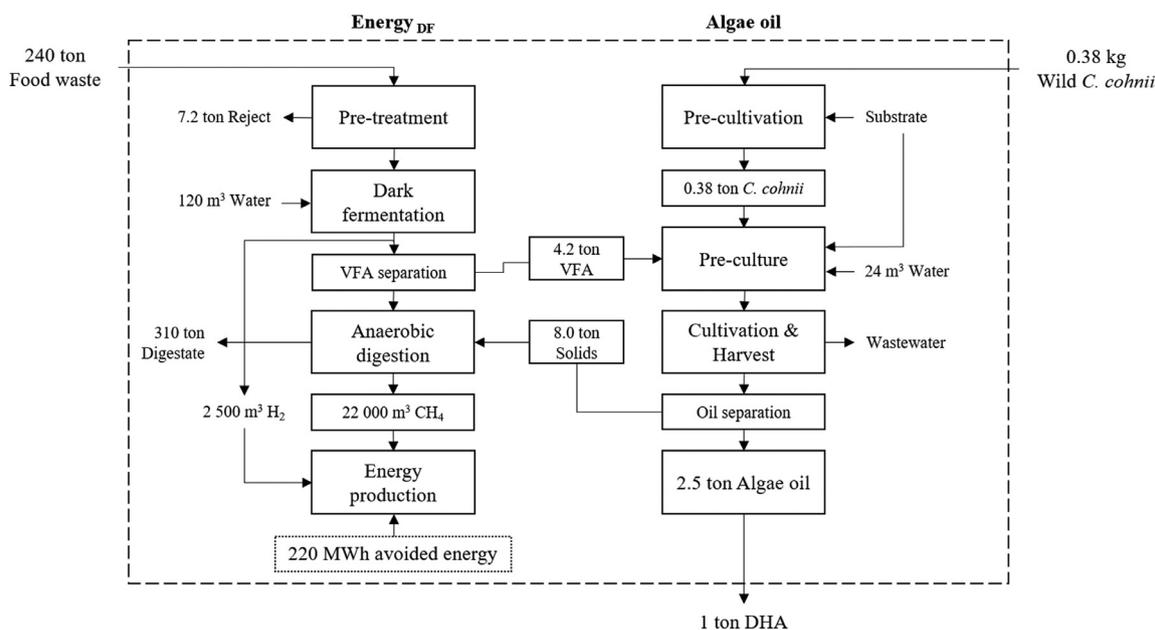


Fig. 2. Illustration of the model set-up for the *Algae scenario*. The dashed line represents the system boundary and the dotted line illustrates electricity and heat produced from biohydrogen and biogas, included via system expansion. Additional electricity, heat and transports are not included in this illustration.

### 3.2.1. Algae scenario

The *Algae scenario* (Fig. 2) process comprised production of *algae oil* via heterotrophic cultivation of *C. cohnii*, using VFA extracted from DF as the primary feedstock, and  $Energy_{DF}$  that represent energy production using the remaining food waste and algae biomass to produce biohydrogen and biogas.

*C. cohnii* are grown in a pre-cultivation process that requires an algae biomass stock, nutrient substrate, water and electricity as inputs. The substrate required in heterotrophic cultivation of *C. cohnii* mainly consist of a carbon source, a nitrogen source for the first production phase, and salts (Mendes et al., 2009). The production process is usually divided into an initial phase of cell growth and a subsequent phase of nitrogen limitation, during which DHA is accumulated inside the cell. Adding VFA can increase the DHA yield, especially if fed to the second cultivation phase. 4.2 ton of VFA was required to produce 1 ton of DHA during cultivation, which was considered the limiting factor in this study. Input data for pre-

cultivation were assumed to be similar as heterotrophic cultivation in closed fermenters suggested by Smetana et al. (2017), with a fishing dataset representing wild *C. cohnii* biomass from the ocean. All nutrient inputs were assumed to be transported by road to the bioreactor facility.

The substrate input for the pre-culture, including yeast and salt, to heterotrophic cultivation was used according to data from previous research (Deprá et al., 2020; Patel et al., 2021; Smetana et al., 2017). Less than 1% of initial *C. cohnii* biomass was used in the pre-culture, which after harvest and dewatering was assumed to make up 20% of the algae culture (200g/L DCW), which is considered the upper limit of reachable values. Since the carbon feedstock was primarily used for biomass growth, the amount of CO<sub>2</sub> emitted from heterotrophic algae cultivation was accounted for by assuming 0.1 kg CO<sub>2</sub> emissions to air per kg algae (Lopes da Silva et al., 2019). As stated by Keller et al. (2017) and Beal et al. (2018), a considerable fraction of water and residues are re-usable. There-

**Table 1**  
Process data for *algae oil* production, expressed per 1 ton DHA.

		Amount	Unit	Ecoinvent dataset <sup>E</sup>
<b>Pre-cultivation</b>				
Input	Wild <i>C. cohnii</i>	$3.8 \times 10^{-1}$	kg	1
	N fertiliser	$7.5 \times 10^{-2}$	kg	2
	P fertiliser	$3.8 \times 10^{-2}$	kg	3
	Glucose	$1.3 \times 10^1$	kg	4
	Water	$3.8 \times 10^{-1}$	m <sup>3</sup>	5
	Natural gas	$7.5 \times 10^{-2}$	kWh	6
	Steam	$1.9 \times 10^1$	kg	7
	Electricity	$6.8 \times 10^0$	kWh	(1)
	Processing facility	$4.7 \times 10^{-7}$	unit	8
	Transport (substrate)	$4.0 \times 10^{-1}$	tkm	9
Output	<i>C. cohnii</i>	$3.8 \times 10^1$	kg	
	CO <sub>2</sub> to air	$3.8 \times 10^0$	kg	10
<b>Pre-culture</b>				
Input	<i>C. cohnii</i>	$3.8 \times 10^{-2}$	ton	
	VFA from DF	$4.2 \times 10^0$	ton	
	Yeast	$7.5 \times 10^{-2}$	ton	11
	Reef salt	$9.4 \times 10^{-1}$	ton	12
	Molasses	$3.4 \times 10^{-1}$	ton	13
	Water	$2.4 \times 10^1$	m <sup>3</sup>	5
	Processing facility	$9.3 \times 10^{-4}$	unit	8
	Transport (substrate)	$5.6 \times 10^3$	tkm	14
Output	Algae culture	$5.4 \times 10^1$	ton	
<b>Cultivation &amp; Harvest</b>				
Input	Algae culture	$5.4 \times 10^1$	ton	
	Electricity	$6.6 \times 10^0$	MWh	(1)
	Heat (re-used)	$8.2 \times 10^{-1}$	MWh	
	Processing facility	$9.3 \times 10^{-4}$	unit	8
Output	CO <sub>2</sub> to air	$1.1 \times 10^0$	ton	10
	Algae suspension	$1.1 \times 10^1$	ton	
	Wastewater	$1.8 \times 10^1$	m <sup>3</sup>	15
<b>Oil separation</b>				
Input	Algae suspension	$1.1 \times 10^1$	ton	
	Electricity	$5.5 \times 10^0$	MWh	(1)
	Heat (re-used)	$9.6 \times 10^{-3}$	MWh	
	Processing facility	$2.2 \times 10^{-6}$	unit	16
Output	Algae oil	$2.5 \times 10^0$	ton	
	Algae solids	$8.0 \times 10^0$	ton	
<b>Transport to Norway</b>				
Input	Transport (algae oil)	$4.7 \times 10^3$	tkm	14
Output	DHA (algae oil)	$1.0 \times 10^0$	ton	

<sup>E</sup>Ecoinvent datasets used in SimaPro, see [Appendix A](#) and [B](#).

fore, a 50% recirculation of wastewater from cultivation and harvest was assumed in this study. Inputs for electricity and heat used for cultivation, harvest, dewatering and oil separation were based on previous studies ([Hosseinzadeh-Bandbafha et al., 2020](#); [Shi et al., 2019](#); [Smetana et al., 2017](#)). The oil separation process involves cell disruption using an oil mill, while additional electricity is used for mechanical pressing and heat was used for pre-treatment, extraction and drying of biomass ([Passell et al., 2013](#)). Mechanical pressing was favoured over chemical means to extract the DHA-containing oleaginous fraction from the biomass since the algae oil was intended for food ([Lopes da Silva et al., 2019](#)). To calculate the amount of algae required per functional unit, a 40% DHA content in algae oil were assumed alongside a biomass-specific DHA content of 10% in *C. cohnii* ([Hillig et al., 2014](#); [Swaaf et al., 2001](#)). The lipid content in *C. cohnii* was set to a maximum of 24% (w/w), as suggested in previous studies ([John, 2009](#); [Mendes et al., 2009](#); [Passell et al., 2013](#)). The remaining solid algae biomass was re-used in AD to produce biogas. All inputs and outputs are presented in [Table 1](#).

Energy<sub>DF</sub> represents the energy production process via DF with VFA extraction and anaerobic digestion ([Fig. 2](#)), during which biogas with 60% CH<sub>4</sub> content and biohydrogen was assumed to be produced. The amount of extractable VFA from DF was set to 18 g VFA/kg FW, which is similar to the AD output suggested by [Paritosh et al. \(2017\)](#). Assuming 97% biodegradable content in

collected food waste, 240 ton were needed to produce 4.2 ton VFA. The 3% of non-biodegradable material was sorted out at pre-treatment and assumed to be equal parts of plastic that was re-used in municipal waste incineration and aluminium for recycling. The values applied for total solids (TS) and volatile solids (VS) in pre-treated food waste were 26% TS and 24% VS, as suggested by [Yi et al. \(2014\)](#) and [Slorach et al. \(2019\)](#). Electricity requirement in pre-treatment was set to 150 kWh/ton TS ([Carlsson, 2015](#); [Pöschl et al., 2010](#)). Construction of the waste preparation facility was included. The total amount of electricity and heat required to produce biogas was similar to that in [Opatokun et al. \(2017\)](#), but it was assumed that 90% of total energy consumption was used during DF (mixing and pumping) and the remaining 10% in AD. Biohydrogen production potential of 45 m<sup>3</sup>/t VS during DF was assumed, which is an average value based on previous studies ([Hou et al., 2020](#); [Pu et al., 2019](#); [Wainaina et al., 2020](#)). The processing unit used for DF and AD was assumed to be an AD plant with methane recovery. Data from a vegetable oil refinery were used to assess the VFA separation process, where 0.21 kWh electricity per kg extracted VFA was assumed ([Hosseinzadeh-Bandbafha et al., 2020](#)). Solids from algae production were re-used in AD and assumed to have equivalent CH<sub>4</sub> potential to the food waste effluent from DF, although the true value might be higher. The energy production process included energy and processing facilities for 40% electricity and 50% heat ([Hakawati et al., 2017](#)) from biogas, when assuming

**Table 2**  
Process data for Energy<sub>DF</sub> production, expressed per 1 ton DHA.

Pre-treatment	Amount	Unit	Ecoinvent dataset <sup>E</sup>
Input	Food waste (FW)	$2.4 \times 10^2$	ton
	Electricity	$9.4 \times 10^0$	MWh (1)
	Waste prep. facility	$4.8 \times 10^{-4}$	unit 17
	Transport (FW)	$7.2 \times 10^3$	tkm 18
	Transport (reject)	$2.2 \times 10^2$	tkm 14
	Output	Pre-treated FW	$2.3 \times 10^2$
Metal reject		$3.6 \times 10^0$	ton 19
Plastic reject		$3.6 \times 10^0$	ton 20
<b>Dark fermentation</b>			
Input	Pre-treated FW	$2.3 \times 10^2$	ton
	Water	$1.2 \times 10^2$	m <sup>3</sup> 5
	Heat (re-used)	$6.6 \times 10^0$	MWh
	Electricity	$2.1 \times 10^3$	MWh (1)
	Processing facility	$1.9 \times 10^{-3}$	unit 21
Output	Slurry from DF	$4.7 \times 10^2$	ton
	Hydrogen (H <sub>2</sub> )	$2.5 \times 10^3$	m <sup>3</sup>
<b>VFA extraction</b>			
Input	Slurry from DF	$4.7 \times 10^2$	ton
	Electricity	$8.8 \times 10^{-1}$	MWh (1)
	Oil processing facility	$4.6 \times 10^{-7}$	unit 22
Output	VFA	$4.2 \times 10^0$	ton
	Effluent from DF	$3.4 \times 10^2$	ton
<b>Anaerobic digestion</b>			
Input	Effluent from DF	$3.4 \times 10^2$	ton
	Algae solids	$8.0 \times 10^0$	ton
	Heat (re-used)	$3.5 \times 10^0$	MWh
	Electricity	$2.4 \times 10^{-1}$	MWh (1)
	Processing facility	$1.0 \times 10^{-2}$	unit 21
	Transport (algae solids)	$8.0 \times 10^1$	tkm 9
	Transport (digestate)	$6.1 \times 10^3$	tkm 14
Output	Methane (CH <sub>4</sub> )	$2.2 \times 10^4$	m <sup>3</sup>
	Digestate	$3.1 \times 10^2$	ton
<b>Energy production</b>			
Input	Hydrogen (H <sub>2</sub> )	$2.5 \times 10^3$	m <sup>3</sup>
	Methane (CH <sub>4</sub> )	$2.2 \times 10^4$	m <sup>3</sup>
	CHP facility	$1.0 \times 10^2$	unit 23
Output	Electricity	$1.0 \times 10^2$	MWh
	Heat	$1.2 \times 10^2$	MWh
	Heat (internal use)	$1.1 \times 10^1$	MWh
<b>System expansion</b>			
	Avoided electricity	$1.0 \times 10^2$	MWh (2)
	Avoided heat	$1.2 \times 10^2$	MWh 24

<sup>E</sup>Ecoinvent datasets used in SimaPro, see [Appendix A](#) and [B](#).

a 90% overall efficiency rate in combined heat and power (CHP) production. The input was calculated based on the amount of electricity generated. Standard values for the energy content in H<sub>2</sub> and CH<sub>4</sub> were used (2.99 and 9.97 kWh/m<sup>3</sup>, respectively). Digestate production was calculated assuming 50% water re-circulation in AD. The main output was 1 ton DHA from algae oil, while the by-products electricity and heat from biohydrogen and biogas were included via system expansion as avoided energy. Inputs and outputs are presented in [Table 2](#).

### 3.2.2. Fish scenario

The process for the *Fish scenario* ([Fig. 3](#)) comprised production of *fish oil* in reduction fisheries using wild Peruvian anchovy, and Energy<sub>AD</sub> describing energy production using the same amount of food waste as was available in the *Algae scenario* to produce biogas in Germany. A 10% DHA content was assumed for Peruvian anchovy oil ([Sissener, 2018](#)). The amount of wild anchovy required to produce 10 ton fish oil was based on data in [Silva et al. \(2018\)](#). Transport from Lima to Norway via the harbour in Venezuela was included. Inputs and outputs are presented in [Table 3](#).

Production of Energy<sub>AD</sub> assumed the same inputs and processing calculations as described for Energy<sub>DF</sub>, but without DF, VFA extraction and addition of algae solids. [Table 4](#) illustrate the process data used for Energy<sub>AD</sub> production. The main output from this sce-

nario was 1 ton DHA from fish oil, while the by-product biogas (subsequently used for electricity and heat) was included via system expansion.

### 3.3. Life cycle impact assessment

Life cycle impact assessment translates emissions and resource use to environmental impact at either midpoint or endpoint level for selected impact categories. The midpoint and endpoint approaches are complementary, but the midpoint approach has lower modelling uncertainty and a stronger relation to the environmental impact, while the endpoint approach provides a better indication of the environmental relevance ([Huijbregts et al., 2016](#)). In general, characterisation at midpoint has lower uncertainty and a stronger relation to the elementary flows than endpoint characterization, but characterization at endpoint can provide a better indication of the environmental relevance of the flows. The ReCiPe 2016 method includes 18 midpoint categories that can be directly translated into endpoint impact, using a constant characterisation factor (CF) for each impact category. This is done by assuming that all stressors are identical after midpoint impact. The endpoint indicators can be aggregated into a common unit to describe the impact for an area of protection, e.g., Ecosystem damage that consists of 13 endpoint indicators expressed in the unit *species per*

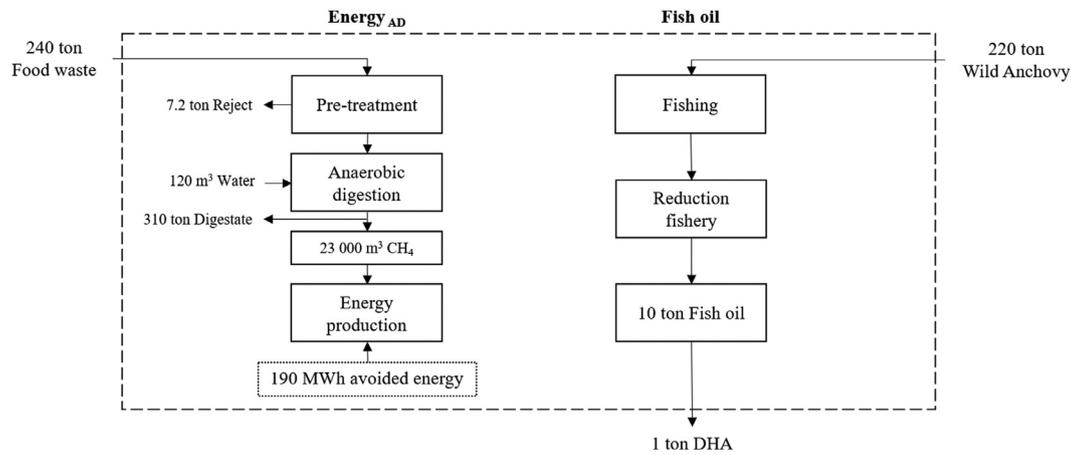


Fig. 3. Illustration of the model set-up for the *Fish scenario*. The dashed line represents the system boundary and the dotted line illustrates the electricity and heat produced from biogas, included via system expansion. Additional electricity, heat and transport are not included. AD: anaerobic digestion, DHA: docosahexaenoic acid.

**Table 3**  
Process data for *fish oil* production, expressed per 1 ton DHA.

		Amount	Unit	Ecoinvent dataset <sup>E</sup>	
<b>Fishing</b>					
Input	Wild anchovy	$2.2 \times 10^2$	ton		
	Fishing process	$2.1 \times 10^2$	ton	25	
Output	Landed anchovy	$2.1 \times 10^2$	ton		
	Discarded fish	$8.7 \times 10^0$	ton		
<b>Reduction fishery</b>					
Input	Landed anchovy	$2.1 \times 10^2$	ton		
Output	Processing facility	$1.0 \times 10^1$	ton	26	
	Fish oil	$1.0 \times 10^1$	ton		
<b>Transport to Norway</b>					
Input	Transport (fish oil)	$4.3 \times 10^4$	tkm	27	
		$8.1 \times 10^4$	tkm	28	
	Output	DHA (fish oil)	$2.3 \times 10^3$	tkm	14
			$1.0 \times 10^0$	ton	

<sup>E</sup>Ecoinvent datasets used in SimaPro, see Appendix A.

**Table 4**  
Process data for *Energy<sub>AD</sub>* production, expressed per 1 ton DHA.

		Amount	Unit	Ecoinvent dataset <sup>E</sup>
<b>Pre-treatment</b>				
Input	Food waste (FW)	$2.4 \times 10^2$	ton	
	Electricity	$9.4 \times 10^0$	MWh	(1)
	Waste prep. facility	$4.8 \times 10^{-4}$	unit	17
	Transport (FW)	$7.2 \times 10^3$	tkm	18
	Transport (reject)	$2.2 \times 10^2$	tkm	14
Output	Pre-treated FW	$2.3 \times 10^2$	ton	
	Metal reject	$3.6 \times 10^0$	ton	19
	Plastic reject	$3.6 \times 10^0$	ton	20
<b>Anaerobic digestion</b>				
Input	Pre-treated FW	$2.3 \times 10^2$	ton	
	Water	$1.2 \times 10^2$	m <sup>3</sup>	5
	Heat (re-used)	$3.7 \times 10^1$	MWh	
	Electricity	$2.3 \times 10^0$	MWh	(1)
	Processing facility	$1.1 \times 10^{-2}$	unit	21
	Transport (digestate)	$6.2 \times 10^3$	tkm	14
Output	Methane (CH <sub>4</sub> )	$2.3 \times 10^4$	m <sup>3</sup>	
	Digestate	$3.1 \times 10^2$	ton	
<b>Energy production</b>				
Input	Methane (CH <sub>4</sub> )	$2.3 \times 10^4$	m <sup>3</sup>	
	CHP processing facility	$1.0 \times 10^2$	unit	23
Output	Electricity	$1.0 \times 10^2$	MWh	
	Heat	$9.0 \times 10^1$	MWh	
	Heat (internal use)	$3.7 \times 10^1$	MWh	
<b>System expansion</b>				
Output	Avoided electricity	$1.0 \times 10^2$	MWh	(2)
	Avoided heat	$9.0 \times 10^1$	MWh	24

<sup>E</sup>Ecoinvent datasets used in SimaPro, see Appendix A and B.

year (species.yr). This unit can be interpreted as the potential disappeared fraction of species each year. Species in this method include plants and microorganisms that support higher terrestrial and aquatic trophic levels of the food chain (Crenna et al., 2020). Loss of these species will affect the food chain, but the direct disappearance of higher organisms or the impact on endangered species are not included in the ReCiPe method. As concluded in a review by Ruiz-Salmón et al. (2021), the most frequently assessed impact categories for seafood at midpoint are global warming, acidification and eutrophication, while Scherer et al. (2020) also emphasise the importance of including land use and sea use impact when addressing food security and biodiversity conservation. These indicators (climate change, pollution and change in habitat) has also been identified as key contributors to biodiversity loss and damage to ecosystem quality at endpoint level (Díaz et al., 2019; Marques et al., 2021; Scherer et al., 2020). Therefore, this study assesses global warming, terrestrial acidification, freshwater eutrophication and land use at midpoint level. To enable a more holistic assessment, which is especially important for systems dependent on inputs from the biosphere, this study also consider damage to ecosystem quality as an complementary assessment at endpoint level (Huijbregts et al., 2016). SimaPro 9 was used to model the system and ReCiPe 2016 was used to assess impact at midpoint and endpoint, which ensured compatibility with established LCIA methods.

### 3.4. Sensitivity analysis

Four sensitivity analyses were performed to identify the influence of uncertainties on the results, focusing on future energy development, optimised VFA production, increased energy demand and biotic resources. By changing one parameter at a time, the uncertainty related to data and assumption could be quantified. The first sensitivity analysis reflected the estimated future energy development, aiming to reduce the dependency on fossil fuels and reach the long-term goal of 100% renewable energy (Bosell et al., 2017). Renewables including wind and solar power are projected to dominate future power generation (Newell et al., 2020). If electricity and heat produced from biomass primarily replaced renewable energy, some benefits attributed to biogas might shift. Therefore, future energy development was simulated by substituting the energy included via system expansion with a potential future German energy mix (Table B.1 in Appendix B). According to the United Nations (2019), an optimised production process can promote sustainable use of resources and enhance energy efficiency. The second sensitivity analysis was based on the VFA efficiency assumptions during DF of food waste. Previous research suggests that DF can be optimised to produce up to 25 g VFA/L household food waste, equalling about 5% VFA per unit food waste (Herrero Garcia et al., 2018; Strazzer et al., 2018). Optimised VFA production was simulated by recalculating input and output data in Energy<sub>DF</sub> assuming that 5 g VFA/kg FW could be extracted. The increased efficiency meant that 84 ton pre-treated food waste was required to produce 4.2 ton VFA, which was also accounted for in Energy<sub>AD</sub>.

Another important aspect to consider is the energy inputs in the *Algae scenario*, especially for algae oil production. This technique is still under development, thus making assumptions regarding the actual energy demand in a large-scale production uncertain. To quantify this uncertainty, all electricity and heat inputs in Table 1, alongside electricity required for VFA separation in Table 2, was increased with 20% to simulate a more energy intensive algae process. The final sensitivity analysis considered the uncertainty of biotic resource depletion in LCA. One aspect of the biodiversity impact is biomass removal from aquatic ecosystems, which affects ecosystems both in terms of resource impact due to the altered stock level of the species and impacts on life support functions. In a

study on sea use impact, Langlois et al. (2015) developed CF for impact on life support functions considering the amount of removed biomass of a certain species. A life support function scenario was simulated using the suggested CF for life support function, where the mass of wild algae removed from ocean in Table 1 was multiplied by 1.4 and the mass of wild anchovy removed from ocean in Table 3 by 13.4.

### 3.5. Product LCA

Scenario analysis is a valuable tool for evaluating commercial and conceptual system set-ups, while also enabling comparison between the scenarios. However, to increase the applicability of the assessed results outside the modelled scenarios, a product LCA perspective can be applied. Product LCA considers an impact related to a specific product, which facilitates comparison with similar products and in turn can increase the benchmarking properties of the result. The product LCA approach used in this study was conducted by subtracting the environmental impact for Energy<sub>AD</sub> in both scenarios. Thereby, only the net environmental impact for producing VFA and *algae oil* instead of *fish oil* and energy was assessed. The product LCA assessed the climate impact (kg CO<sub>2</sub>eq) per ton DHA and per kg oil, to enable comparison with similar commercial products. In recent studies, the potential to replace DHA in fish feed with DHA from different vegetable sources has been investigated. The climate impact of DHA from the *Algae scenario* and the *Fish scenario* can be compared with similar products, such as Canola or linseed oil. The assessed impact can then be evaluated in comparison with Canola oil and linseed oil, two vegetable oils with high DHA potential that could be used to replace DHA in fish feed (Bélanger-Lamonde et al., 2018; Petrie et al., 2020; Zhou et al., 2019). A 13% bioavailable DHA content was assumed for Canola oil (Petrie et al., 2020), while an ALA content of 56% was assumed for linseed oil (Burns-Whitmore et al., 2019; Karapanagiotidis et al., 2007). Assuming 1% conversion of ALA to DHA in fish, the potential DHA in linseed oil was calculated to be 0.6%. A maximum climate impact for canola rapeseed oil was set to 2.5 kg CO<sub>2</sub>eq per L oil (Röös, 2012). Assuming 0.75 kg CO<sub>2</sub>eq per kg linseed (Nemecek et al., 2012) and 40% oil content, the maximum climate impact for linseed oil was calculated to be 1.9 kg CO<sub>2</sub>eq per kg oil.

## 4. Results

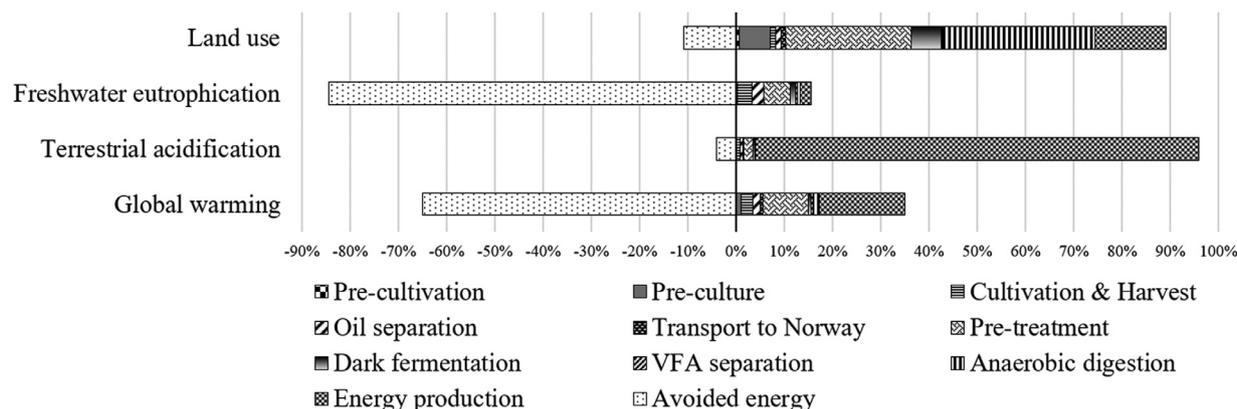
### 4.1. Environmental impact

The results showed that for every ton DHA produced in the *Algae scenario*, 14 of 18 midpoint impacts assessed had lower environmental impact compared to the *Fish scenario*, including global warming, terrestrial acidification, and land use (see Table C.1 in Appendix C). As illustrated in Table 5, both scenarios had negative values for global warming and freshwater eutrophication, which means that for every ton DHA produced, the environmental impact was mitigated.

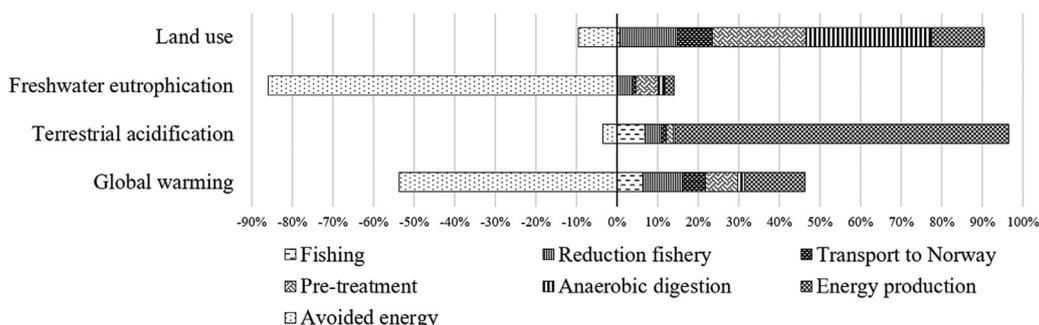
Inclusion of by-products via system expansion was the main reason for the mitigated environmental impact in both the *Algae scenario* (Fig. 4) and the *Fish scenario* (Fig. 5). For global warming and freshwater eutrophication, the highest environmental impact in both scenarios occurred during the pre-treatment of food waste, while the most contributing process for terrestrial acidification and land use was energy production and anaerobic digestion, respectively. *Algae oil* processing caused only about 5% of the total global warming impact for the *Algae scenario*, 10% of land use impact, 4% of terrestrial acidification and 6% of freshwater eutrophication impact. The *fish oil* process caused about 22% of the global warming and land use impact, while about 12% of terrestrial acidification

**Table 5**  
Environmental impact per 1 ton DHA.

	Global warming kg CO <sub>2</sub> eq	Terrestrial acidification kg SO <sub>2</sub> eq	Freshwater eutrophication kg Peq	Land use m <sup>2</sup> a crop eq
<b>Algae scenario</b>	$-5.2 \times 10^4$	$3.5 \times 10^3$	$-9.4 \times 10^1$	$2.7 \times 10^3$
<b>Fish scenario</b>	$-1.5 \times 10^4$	$3.9 \times 10^3$	$-9.7 \times 10^1$	$3.2 \times 10^3$



**Fig. 4.** Environmental impact of the *Algae scenario*, illustrating the percentage of total impact for each production process



**Fig. 5.** Environmental impact of the *Fish scenario*, illustrating the percentage of total impact for each production process.

and 5% of freshwater eutrophication originated from it. The fishing process contributed less than 1% of the total land use impact in the *Fish scenario* (Fig. 5).

#### 4.2. Ecosystem damage

The results showed a lower impact for the *Algae scenario* than the *Fish scenario* for nine of 12 endpoint categories assessed, including global warming, terrestrial acidification and land use (see Table C.2 in Appendix C). Total Ecosystem damage per ton DHA produced in the *Algae scenario* was  $5.5 \times 10^{-4}$  species.yr, the *Fish scenario* inferred  $8.1 \times 10^{-4}$  species.yr. The results also showed that *algae oil* and *fish oil* caused Ecosystem damage of  $5.1 \times 10^{-5}$  and  $3.1 \times 10^{-4}$  species.yr, respectively, while the value for both Energy<sub>DF</sub> and Energy<sub>AD</sub> was  $5.0 \times 10^{-4}$  species.yr. Similar to the results at midpoint level, *algae oil* processing caused about 10% of the Ecosystem damage, while *fish oil* process caused about 18% (Fig. 6). This suggests that inclusion of by-products had a considerable effect on the total impact on ecosystem quality and effect on biodiversity. As the results illustrated in Fig. 7 illustrate, the largest impact at endpoint level for both scenarios was terrestrial acidification, global warming, freshwater eutrophication and land use. A negative value for Ecosystem damage indicates mitigation of disappeared species per year, whereas a higher positive value can be interpreted as less favourable for biodiversity.

#### 4.3. Sensitivity analysis and product LCA

The results from the sensitivity analysis at midpoint level showed that the *Algae scenario* inferred lower terrestrial acidification impact in comparison to *Fish scenario* even when future energy development, optimised VFA production, increased energy demand and impact on life support function was simulated (Table 6). The *Algae scenario* inferred lower global warming potential for future energy and life support function, but higher global warming potential than the *Fish scenario* for optimised VFA and increased energy. Land use at midpoint was also higher for *Algae scenario* when life support function was simulated.

Even though the global warming potential and land use impact respectively increased for the *Algae scenario* when increased energy and life support function was simulated, the *Fish scenario* still inferred a higher damage to ecosystem quality for all sensitivity analyses performed (Fig. 8). Ecosystem damage for the *Algae* and *Fish scenarios* increased when future energy production was simulated and decreased when optimised VFA content was assumed. When effects on biotic resources were included via life support functions, the impact for the *Fish scenario* increased markedly, while the Ecosystem damage for the *Algae scenario* remained similar to that in the base case.

The product LCA results showed that the climate impact per ton oil was higher for algae oil than for Canola and linseed oil, but lower than for fish oil (Table 7). Algae oil had the lowest impact

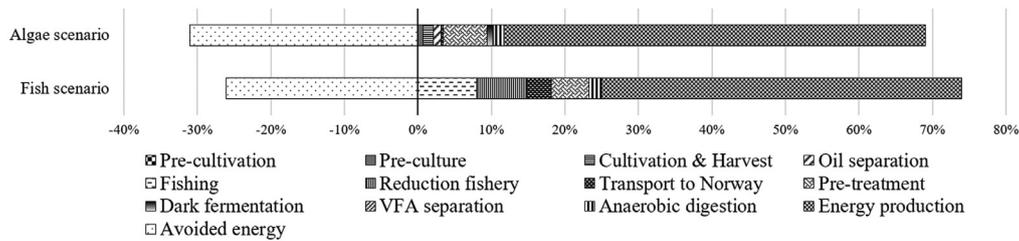


Fig. 6. Ecosystem damage for the Algae scenario and Fish scenario, illustrating the percentage of total impact for each process.

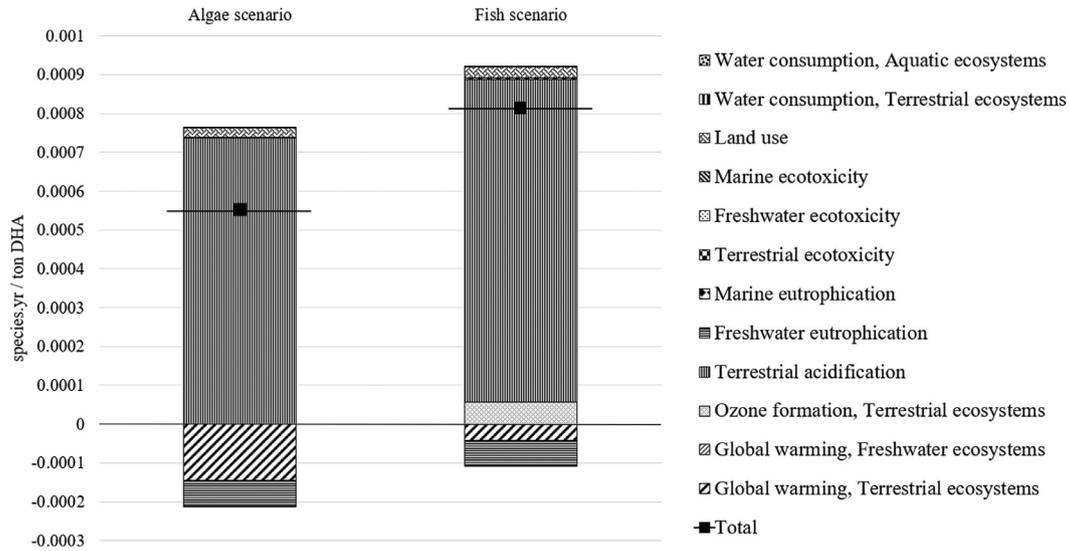


Fig. 7. Ecosystem damage in (left) the Algae scenario and (right) the Fish scenario, illustrating the contribution from each endpoint indicator.

Table 6

Sensitivity analysis result at midpoint level, expressed per 1 ton DHA.

Algae scenario	Global warming kg CO <sub>2</sub> eq	Terrestrial acidification kg SO <sub>2</sub> eq	Freshwater eutrophication kg Peq	Land use m <sup>2</sup> a crop eq
Future energy	2.9 × 10 <sup>4</sup>	3.1 × 10 <sup>3</sup>	-2.0 × 10 <sup>-5</sup>	-6.3 × 10 <sup>-5</sup>
Optimised VFA	-1.0 × 10 <sup>4</sup>	1.3 × 10 <sup>3</sup>	-3.0 × 10 <sup>1</sup>	1.4 × 10 <sup>3</sup>
Increased energy	3.0 × 10 <sup>4</sup>	3.1 × 10 <sup>3</sup>	-9.8 × 10 <sup>1</sup>	1.2 × 10 <sup>3</sup>
Life support func.	-5.2 × 10 <sup>4</sup>	3.5 × 10 <sup>3</sup>	-9.4 × 10 <sup>1</sup>	2.7 × 10 <sup>3</sup>
Fish scenario	Global warming kg CO <sub>2</sub> eq	Terrestrial acidification kg SO <sub>2</sub> eq	Freshwater eutrophication kg Peq	Land use m <sup>2</sup> a crop eq
Future energy	6.7 × 10 <sup>4</sup>	3.6 × 10 <sup>3</sup>	-2.1 × 10 <sup>-5</sup>	-4.8 × 10 <sup>-5</sup>
Optimised VFA	-1.5 × 10 <sup>4</sup>	1.7 × 10 <sup>3</sup>	-3.1 × 10 <sup>1</sup>	1.8 × 10 <sup>3</sup>
Increased energy	-1.5 × 10 <sup>4</sup>	3.9 × 10 <sup>3</sup>	-9.7 × 10 <sup>1</sup>	3.2 × 10 <sup>3</sup>
Life support func.	2.3 × 10 <sup>5</sup>	7.2 × 10 <sup>3</sup>	-7.2 × 10 <sup>1</sup>	1.8 × 10 <sup>3</sup>

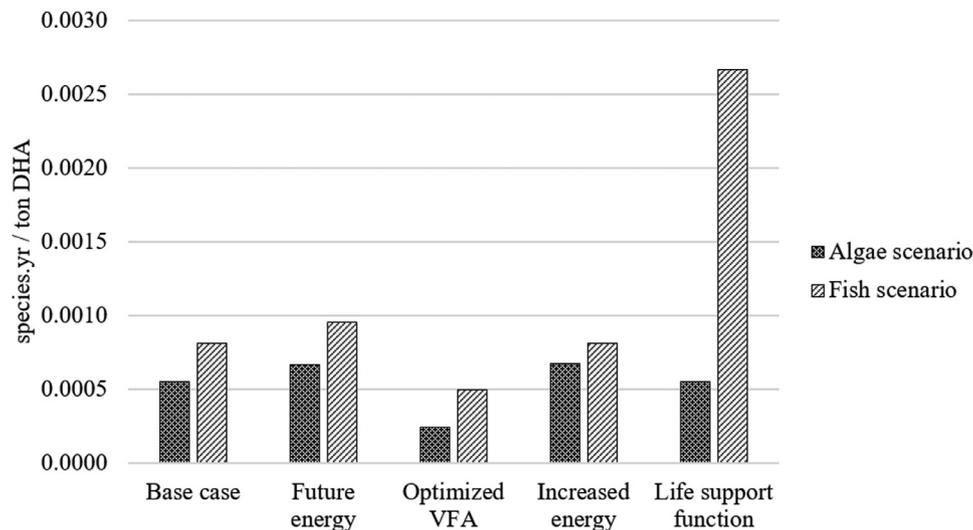


Fig. 8. Results of sensitivity analysis for Ecosystem damage with respect to the Algae scenario and the Fish scenario.

**Table 7**  
Climate impact (product perspective), expressed per ton DHA and per kg oil.

	ton CO <sub>2</sub> eq/ton DHA	kg CO <sub>2</sub> eq/kg oil
Algae oil <sup>1</sup>	7.6	3.0
Fish oil <sup>1</sup>	44	4.4
Canola oil <sup>2</sup>	23	2.3
Linseed oil <sup>2</sup>	330	1.9

<sup>1</sup>Bioavailable DHA

<sup>2</sup>ALA converted to DHA

per ton of DHA produced, while linseed oil and fish oil had the highest impact in this regard.

## 5. Discussion

### 5.1. Environmental impact of DHA from algae oil and fish oil

One of the most important findings in this study was that the environmental impact per ton DHA produced was lower in the *Algae scenario* than in the *Fish scenario* with respect to global warming, terrestrial acidification, land use and Ecosystem damage (Table 5 and Fig. 7). In addition, 20 MWh more electricity and heat was produced in the *Algae scenario*. This, together with overall lower emissions from *algae oil* production compared with *fish oil* production, was the main reason for the lower environmental impact. *Algae oil* production was also less dependent on fossil fuels and electricity, which could explain the lower impact for global warming, terrestrial acidification and eutrophication. Use of a constant CF from midpoint to endpoint likely explains why the climate impact, terrestrial acidification and freshwater eutrophication also contributed most to the impact at endpoint level. As illustrated in Table 5, the impact on water consumption and terrestrial ozone formation was more than 5-fold and 20-fold higher, respectively, in the *Fish scenario*. However, since water consumption and terrestrial ozone formation made a low contribution to the endpoint level, their impact was low when comparing products. The climate impact for *fish oil* was about twice that reported by Silva et al. (2018), owing to a higher contribution from fishing and reduction fishery processing in this study. The difference in climate impact might be explained by different electricity inputs or the updated Ecoinvent datasets, where buildings and machinery are included, which were used to assess impacts in this study. Moreover, most transport in this study was assumed to be of Euro class IV, which reduced the climate impact from transport. The dependency on fossil fuels used for fishing and reduction fishery processing has previously been identified as a contributing factor for the environmental impact of fish oil (Avadí and Fréon, 2013; Cashion et al., 2017; Pelletier and Tyedmers, 2007). Therefore, the impact determined for *fish oil* can be considered consistent with previous findings.

When evaluating the result from this study with previous research it is important to emphasise that all LCA results are highly dependent on given assumptions and methodological choices. Comparing numerical results should thus be done with this in mind, especially regarding impact for the *Algae scenario* since less data and previous LCA research has covered this topic. However, some previous studies have included similar processes as assessed in this study. In a study by Deprá et al. (2020), the environmental impact of multiple algae species, including *C. cohnii* cultivation using glucose as primary carbon feedstock, was assessed. They used a similar method and process for algae cultivation and oil separation as assumed in this study, while also including the impact categories global warming, acidification potential, eutrophication potential and land use. Their result showed an environmental impact of about 178 ton CO<sub>2</sub>eq, 0.9 ton SO<sub>2</sub>eq, 0.3 ton PO<sub>4</sub>eq and 900 m<sup>2</sup> per ton DHA produced. If excluding the contribution

from avoided electricity and heat in the *Algae scenario* (Table C.1 in Appendix C), the values for global warming, acidification, eutrophication and land use correspond to 60 ton CO<sub>2</sub>eq, 3.6 ton SO<sub>2</sub>eq, 0.02 ton PO<sub>4</sub>eq and 3000 m<sup>2</sup> per ton DHA. The main difference between the results presented by Deprá et al. (2020) and the results in this study is primarily with respect to global warming and land use. The higher value for global warming is likely caused by the different inputs, such as glucose as primary carbon feedstock and global average instead of the current German electricity mix, while the higher land use is likely a consequence of inclusion of buildings in this study. Even though the numerical results are not perfectly comparable due to different assumptions, the method and overall results can be considered supportive of each other.

Similarly, Schade et al. (2020) conducted a comparative LCA study on fatty acids and protein from microalgae and fish, also including the same midpoint indicators as this study. Several algae species were studied for their potential to accumulate DHA and EPA, including the algae *Phaeodactylum tricornutum*. By calculating an average impact value from their published supplementary material, result shows an impact of about 2.4 kg CO<sub>2</sub>eq, 7.2 g SO<sub>2</sub>eq, 8.8 g PO<sub>4</sub>eq and 0.17 m<sup>2</sup> per kg dry algae biomass. If assuming a 4% DHA content in the dry biomass from *Phaeodactylum tricornutum*, the resulting midpoint impact with respect to climate impact, acidification, eutrophication and land use was roughly 60 ton CO<sub>2</sub>eq, 0.18 ton SO<sub>2</sub>eq, 0.22 ton PO<sub>4</sub>eq and 4300 m<sup>2</sup> per ton DHA respectively. The result suggested by Schade et al. (2020) are very close to the environmental impact assessed at midpoint level in this study, when excluding the contribution from the avoided electricity and heat in the *Algae scenario*, even though a different microalgae species and thereby cultivation method was used. To our knowledge, no previous study on algae cultivation has included endpoint indicators for ecosystem quality. Therefore, a comparison with previous studies was not possible even though this aspect is highly requested. The results from this study can thus serve as a basis for future comparisons and enable an improved availability in the emerging field of industrial DHA and EPA production with microalgae. By inclusion of biotic resources, this study also provides a basis for a more holistic future development analysis, where both midpoint and endpoint indications are included in the LCA assessment.

### 5.2. Environmental impact of energy production and product LCA

Energy production, and the corresponding avoided environmental burden from using the biohydrogen and biogas produced to substitute the German electricity mix, had a considerably high influence on both midpoint and endpoint indicators for the *Algae* and *Fish scenario* (Figs. 4–6). A strong influence of including by-products was also identified in a study by Elginöz et al. (2020), who saw a similar trend when assessing innovative food waste management systems where VFA and methanol were produced. Our results suggested that energy processing was the main contributing factor to terrestrial acidification, while avoided use of lignite for electricity production was the main cause of mitigated freshwater eutrophication, at both midpoint and endpoint level. The main cause of terrestrial acidification during energy production was likely emissions of NO<sub>x</sub>, NH<sub>3</sub> or SO<sub>2</sub> to air (Huijbregts et al., 2016; Whiting and Azapagic, 2014), caused by the dataset used to describe CHP process of biogas. The avoided use of lignite as an electricity source likely mitigated freshwater eutrophication by reducing phosphorus and nitrogen emissions to soil, air and water (Wang et al., 2015). This is likely the cause for the negative freshwater eutrophication (Table 5). Even though the high influence of terrestrial acidification and freshwater eutrophication on the outcome was unexpected, the same dataset was used

in both Energy<sub>DF</sub> and Energy<sub>AD</sub>, and thereby the same uncertainty applied to both scenarios.

The results for product LCA, where only the net environmental burden of avoided energy was assigned to the *Algae scenario*, showed that algae oil had a lower climate impact per ton DHA produced in comparison to fish oil, canola oil and linseed oil (Table 7). It is important to note, however, that both algae oil and fish oil contain bioavailable DHA, while Canola and linseed oil require conversion of ALA to provide DHA. Conversion rate of ALA to DHA can vary greatly, so the actual DHA per ton oil for canola oil and linseed oil shows a large range, meaning that direct comparisons with algae oil and fish oil should be performed with caution. However, both canola oil and algae oil have established potential to replace fish oil in fish feed (Bélanger-Lamonde et al., 2018; Cottrell et al., 2020; Petrie et al., 2020).

### 5.3. Uncertainties

According to Hetherington et al. (2014), the parameter uncertainties are often higher for processes using emerging technologies and early-stage LCAs, where production, inputs and outputs have not yet been fully established. The *Algae scenario* can be considered an emerging technology, for which the accessibility of inventory data was the main source of uncertainty. For instance, Ecoinvent 3.5 contains data on fishing activities and Peruvian site locations (Avadí et al., 2020), but datasets for algae aquaculture, DF and VFA separation are currently not available. Similar uncertainty was associated with the results for Ecosystem damage and potential loss of biodiversity, where the numerical results, especially for the *Fish scenario*, were likely strongly underestimated. Although, the removal of primary producers could cause damage to higher trophic levels, which should be considered in future assessments. Moreover, the LCI for Peruvian anchovy represents sustainable fishing, even though unsustainable fishing is an established problem (Fréon et al., 2017). If the anchovy used for fish oil were sourced from unsustainable fishing, the Ecosystem damage for the *Fish scenario* would be higher than reported in this study. A final uncertainty aspect was the representation of species.yr in the ReCiPe endpoint approach, where the same weight is assigned to all species of plants and lower organisms, thus not accounting for endangered or overexploited species. Due to the uncertainty, the impact on biodiversity at endpoint level should primarily be used to identify hotspots for actions to reduce environmental burden.

The results from the sensitivity analysis suggested that AD of food waste to produce biogas for electricity and heat can be less favourable as a valorisation method including the material use in the future (Fig. 8), since the energy can no longer replace fossil energy sources. This is an important finding for future developments within food waste management, especially since the method for re-use should be consistent with the most efficient option to optimise resource recovery (Teigiserova et al., 2020). Another important consideration is that the results show the impact primarily using German energy mix and Euro IV road transport, which provide a site-specific result and limited transport emissions. The decreased environmental impact when less food waste was required to produce VFA was likely mainly due to the reduced amount of energy and building required for processing. Based on previous research (Barr and Landis, 2018; Keller et al., 2017; Taelman et al., 2013), the *Algae scenario* could be improved by nutrient recycling (e.g. recycled algae culture medium or re-using additional nutrients from VFA), by efficient energy use (e.g. using methane as fuel for transportation) or by upscaling the production process. In comparison, the fishing and reduction fishery process has been optimised and streamlined for decades, and therefore does not offer the same development potential. Since algae cultivation can be sensitive to trace components, there is a considerable uncertainty

related to the inputs and outputs for this process. As the sensitivity result for optimised VFA production and increased energy show (Table 6), the global warming potential increase for *Algae scenario*, while the land use impact increase when life support function was accounted for. The result indicates that depending on the required energy input the result is uncertain. This highlights the importance of further research and development within algae cultivation for oil accumulation to further increase the knowledge and data availability.

The *Fish scenario* resulted in over 3-fold higher Ecosystem damage in comparison with the base case when the life support function was included. Since more biomass of Peruvian anchovy than wild-type *C. cohnii* was required per ton DHA and since anchovy had a higher trophic level, the CF was higher for anchovy. Direct impact translation to species.yr is not yet possible using available LCIA methods, but this result provides a crucial indication of the magnitude of sensitivity related to Ecosystem damage and effects on biodiversity within LCA. The impact of biotic resources have been identified in previous studies (Avadí and Fréon, 2013; Winter et al., 2017; Woods et al., 2016), where the main conclusion was the importance of developing methods that include multiple aspects of biotic resources in LCA. The results from the present study confirm this conclusion. Another important finding in this study was that even though the *Algae scenario* inferred higher impact at midpoint for multiple sensitivity scenarios, the result for Ecosystem damage at endpoint level was consistently lower in comparison to the *Fish scenario* (Fig. 8). Even though a higher uncertainty is related to impact at endpoint level, alongside the relative uncertainty related to impact on biotic resources in LCA, the results provide an important indication of the environmental relevance which is vital for a more holistic assessment in the future.

### 5.4. Future outlook

An important aspect of sustainable development is to ensure that an increasing global population has access to nutritiously valuable food (FAO, 2020, 2019). Threats to food security due to increased temperatures and changes in natural ecosystem functions are likely to emerge as tangible consequences of climate change and loss of biodiversity. According to Avadí et al. (2020) and FAO (2018), the aquaculture sector will likely continue to grow, while natural DHA synthesis by marine microalgae is estimated to decrease due to global warming (Colombo et al., 2020). Therefore, developing new production methods within the food supply chain, preferably with increased resource recovery to reduce the environmental impact and damage to ecosystem quality, can be considered the most urgent global challenge of today. In the near future, it will also become increasingly important to develop alternative ways to produce DHA (Beal et al., 2018; Cottrell et al., 2020; Russo et al., 2021).

Algae oil production has the potential to expand and meet multiple demands of the growing aquaculture sector, while also lowering the burden on wild-caught fisheries and decreasing the use of biotic resources (Barr and Landis, 2018; Ghamkhar and Hicks, 2020) in comparison with traditional fish feed (Taelman et al., 2013). Given the globality of the current supply chain, using bioreactors for algae cultivation enables a local primary production of DHA which likely will require a shorter transport distance and opportunities for an increased degree of self-sufficiency. In a global context, this is especially relevant with respect to global food security and energy production. As suggested by Chalima et al. (2019), the development of microalgae cultivation processes for oil accumulation has so far been slow, primarily due to the relatively high economic costs related to the emerging technology for algae cultivation and harvest. At present, algae oil would therefore likely result in higher economic costs than fish oil

(Sprague et al., 2017; Yarnold et al., 2019), since it requires further development to be implemented in large-scale production. However, since sustainable development requires a shift from a linear to a circular bioeconomy, utilising available resources from other production systems will become increasingly important to meet the needs of future generations. DHA, as produced in the *Algae scenario*, can bridge a future gap between supply and demand, while short-chain carboxylic acids produced alongside biohydrogen and biogas could be integrated into existing infrastructure for common food waste valorisation methods quite rapidly. To promote the evolution of future technologies with lower environmental impact that also require fewer natural resources to produce products with maintained quality and nutrient composition, companies and policy makers must overcome the hurdle of uncertainty related to new innovations. This is also concluded by Deprá et al. (2020), who also stress the importance of abandon current technology to enable a transition to more sustainable solutions. Previous studies indicate that promoting circular resource use will become increasingly important in the future (Jovanovic et al., 2021; Oliver et al., 2020; Russo et al., 2021). One could therefore argue that fish oil in fish feed should be replaced with algae oil. Important to consider however, is that alternative production methods should be carefully assessed to prevent fish oil being replaced with another potentially unsustainable DHA source. The results in this study indicate that algae are a more sustainable source of DHA than fish, but other sources of DHA should also be evaluated, for instance single cell oils derived from yeast or genetically modified vegetable sources. This is especially important given the uncertainty related to LCA for technologies and solutions that are still under development.

Given that quantities required in the *Algae scenario* are 1:1 scalable, only about 2.7% of the globally available food waste generated at households, retail or service sector is required to produce enough VFA via DF to substitute the yearly demand of about 100 000 tons of DHA from fish oil in aquafeed. Even though enough food waste is already available, it is important to consider potential burden shifts when replacing fish oil with algae oil, such as an increased eutrophication potential (Table 6). However, this also illustrate the importance of investing in further development of this technology since it has a large potential to increase resource efficiency and promote both sustainable aquaculture and improved resource recovery within food waste management. Technologies that favour circular flows, with resource recovery and nutrient recycling, could also contribute to several SDGs (Herrero et al., 2020; Teigiserova et al., 2020). To fully assess the impact on SDG fulfilment, new LCA frameworks are currently being developed (Life Cycle Initiative, 2021; Weidema et al., 2020). Although, the results in this study indicate that, compared with fish oil, DHA from the *Algae scenario* could contribute to multiple SDGs. For instance *climate action* by reduced greenhouse gas emissions (SDG 13), *responsible consumption and production* by increased resource efficiency and reduced waste (SDG 12), *zero hunger* by supporting sustainable food production systems (SDG 2), *affordable and clean energy* by increasing the share of renewable energy in the global energy mix (SDG 7), and ultimately mitigate loss of biodiversity and damage to ecosystem quality by reducing acidification, eutrophication and habitat degradation (SDGs 14 and 15). Given the estimated expansion of aquaculture and increased global demand for food rich in DHA, algae-based aquafeeds thus represent an alternative production method using a carbon source derived from already available resources.

### 5.5. Recommendations for future studies

As established by previous studies, these is an urgent need for life cycle assessments and more data to enable environmental assessments and sustainability evaluation of emerging food and

aquaculture technologies, including microalgae cultivation (Avadí et al., 2020; Lopes da Silva et al., 2019). In order to mimic the composition of fish oil better in biotechnologically derived cell suspensions, algae species, e.g. *Schizochytrium* sp., that produce EPA and DHA in a ratio similar to that in fish oil, could be considered (Hart et al., 2021; Sprague et al., 2015). Alternatively, DHA from *C. cohnii* could potentially replace fish oil supplements in human diets. Some hurdles need to be overcome, however. First, bioproduction needs to be further optimised and made robust, e.g. through integration of suitable online monitoring that allows variable utilisation of feedstock while maintaining cell viability and production capacity, e.g. through technologies that capture the single cells (Delvigne et al., 2018). Under current EU animal by-product legislation, DHA from the *Algae scenario* can only be implemented if exclusively vegetable waste is used to produce VFA, which might have an impact on the yield of DF (Strazzera et al., 2018). This requires further investigation and optimisation of such processes based on cell physiology, as only cells with a certain metabolic turnover excrete VFAs (Bockisch et al., 2018). The best option for food waste valorisation should also be examined in more detail, including both small- and large-scale case studies. This is especially important since we are still a long way from a harmonized assessment of food waste management, which makes it difficult to fully assess the future potential for suggested solutions.

When comparing certain production scenarios, direct effects on biodiversity cannot be assessed with established LCIA methods, so future studies would benefit from including this aspect. Since resource depletion, overfishing and invasive species are some of the main threats to loss of marine biodiversity (Woods et al., 2016), these aspects should be included in studies with a marine biodiversity focus. This could potentially be accomplished by implementing CFs for biotic resource depletion (Hélias et al., 2018), overfishing (Emanuelsson et al., 2014) or invasive species (Hanafiah et al., 2013). To support sustainable development and maintain a rich biodiversity, there is an urgent need for robust and extensive impact assessment methods to account for the full impact on biotic resources (Avadí et al., 2020; Asselin et al. 2020; Winter et al., 2017). One could therefore argue that the most important future research needs are to reduce knowledge gaps and to develop LCA methods that cover all five drivers of biodiversity loss.

## 6. Conclusions

This study assessed the environmental impact of a conceptual *Algae scenario* with DHA produced via the microalgae *C. cohnii* grown in a bioreactor cultivation process using VFA extracted from food waste as its main carbon feedstock. The utilization of VFA from dark fermentation enables a combination of bioconversion for a high added-value DHA product via microalgae with renewable energy production. The impact was compared with that of a conventional *Fish scenario* with DHA derived from Peruvian anchovy. Alongside the environmental impact at midpoint level, the important aspect of ecosystem quality at endpoint level was also assessed using Ecosystem damage as indicator for biodiversity loss. The main by-product in both scenarios was electricity and heat, included via system expansion. The global warming, terrestrial acidification, freshwater eutrophication and land use per ton DHA produced in the *Algae scenario* was found to be -52 ton CO<sub>2</sub>eq, 3.5 ton SO<sub>2</sub>eq, -94 kg Peq, 2700 m<sup>2</sup> eq, respectively. In comparison, the impact per ton DHA in the *Fish scenario* was -15 ton CO<sub>2</sub>eq, 3.9 ton SO<sub>2</sub>eq, -97 kg Peq and 3200 m<sup>2</sup> eq. The Ecosystem damage for Algae scenario and Fish scenario was  $5.5 \times 10^{-4}$  and  $8.1 \times 10^{-4}$  species per year respectively. Even though established LCIA methods only assess indirect effects on biodiversity, the *Algae scenario* resulted in lower Ecosystem damage than the *Fish scenario* even

when a future energy development, optimized VFA production, increased energy demand and effects on biotic resources were considered via sensitivity analyses. This study also showed that included by-products and energy production had a high influence on the total environmental burden for both scenarios. From a product LCA perspective, algae oil had the lowest climate impact per ton DHA for all evaluated oils.

At present, coupling DF and subsequent monocultivation for DHA and EPA production is still under development and thus require higher economic investments to enable a large-scale production similar to traditional fish oil processing. However, the production of valuable DHA with lower environmental impact arguably justifies a higher production cost especially since it also provides an improved food waste valorisation solution and a source of renewable energy. The environmental aspect must be considered a key component in both political decision making and company development to fully achieve a sustainable development, as well as enabling a shift from a linear to a circular bioeconomy where available resources are recovered and used in the most efficient way possible. The result from this study emphasise that Algae oil holds a promising potential to increase sustainability within aquaculture, provided that continued development and optimization of the technology and process is enabled through active decision-making, purposeful investments, and further research.

This study showed that DHA produced by microalgae using VFA from DF of food waste can reduce loss of biodiversity and support sustainable production while satisfying increased future demand for DHA within the food supply chain. This could support sustainable development by meeting current needs for DHA without compromising nature's ability to produce this essential fatty acid in the future. The *Algae scenario* approach also enabled increased resource efficiency by recovering nutrients and resources in food waste for value addition. By using agricultural and food industry by-products to produce DHA, overfishing, for example of Peruvian anchovy, could be counteracted and thereby increasing the overall sustainability of aquaculture while maintaining essential ecosystem quality.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A

Ecoinvent datasets used in SimaPro 9 (PhD licence) to model impact for the *Algae scenario* and the *Fish scenario*. Original data generator, dataset name, geographical location and process modelled using the dataset are shown. All datasets were taken from Ecoinvent 3.5 with the allocation cut-off by classification setting.

**Table A.1**  
Ecoinvent datasets used in SimaPro, using the allocation cut-off by classification setting.

Dataset number	Data generator	Dataset name	Location	Used to model:
1	Symeonidis, A.	Market for marine fish	GLO	Wild C. <i>cohnii</i>
2	System	Market for nitrogen fertiliser, as N	GLO	N fertiliser
3	System	Market for phosphate fertiliser, as P2O5	GLO	P fertiliser
4	System	Market for glucose	GLO	Glucose
5	System	Market group for tap water	RER	Water
6	Faist E., M.	Market for natural gas, high pressure	DE	Natural gas
7	System	Market for steam, in chemical industry	GLO	Steam
8	Dux, D.	Liquid manure storage and processing facility	CH	Algae processing
9	Valsasina, L.	Market for transport, freight, lorry 3.5-7.5 metric ton, EURO4	RER	Transport
10	PRé Consultants	Carbon dioxide, unspecified	n.a.	CO <sub>2</sub> to air
11	System	Market for fodder yeast	GLO	Yeast
12	System	Market for sodium chloride, brine solution	GLO	Reef salt
13	System	Market for molasses, from sugar beet	GLO	Molasses
14	Valsasina, L.	Market for transport, freight, lorry 7.5-16 metric ton, EURO4	RER	Transport
15	System	Market for wastewater, average	Europe without Switzerland	Wastewater
16	Gnansounou, E.	Oil mill construction	CH	Oil separation
17	Kägi, T.	Waste preparation facility construction	CH	FW processing
18	Doka, G.	Market for municipal waste collection service, by 21 metric ton lorry	CH	Transport
19	PRé Consultants	Recycling of aluminium	GLO	Metal reject
20	Treyer, K.	Treatment of municipal solid waste, incineration	DE	Plastic reject
21	Schleiss, K.	Anaerobic digestion plant construction, agriculture, with methane recovery	CH	DF and AD

(continued on next page)

**Table A.1** (continued)

Dataset number	Data generator	Dataset name	Location	Used to model:
22	System.	Market for vegetable oil refinery	GLO	VFA processing
23	Treyer, K. Ruiz, EM.	Heat and power co-generation, biogas, gas engine Excluding contribution from: market for biogas	DE RoW	CHP plant
24	Treyer, K.	Heat and power co-generation, natural gas, combined cycle power plant, 400MW electrical	DE	Heat (fossil)
25	Avadi, A.	Anchovy, capture by steel purse seiner and landing whole, fresh	PE	Fishing anchovy
26	Avadi, A.	Fishmeal and fish oil production, 63–65% protein	PE	Reduction fishery
27	Simons, A.	Transport, freight, lorry 7.5–16 metric ton, EURO4	RoW	Transport
28	System	Market for transport, freight, sea, transoceanic ship with reefer, cooling	GLO	Transport

## Appendix B

In 2020, about 50% of German electricity was generated from renewable sources (Energy-Charts, 2020), while energy use for heat was dominated by fossil fuels (Euroheat & Power, 2019; IEA, 2020). To accurately represent the use of electricity, a custom energy mix was created in SimaPro 9 using Ecoinvent 3.5 datasets. Current electricity mix represent the 2020 production mix, while the future electricity mix consist of entirely renewable energy. The fossil energy dataset is used to assess the avoided energy in Algae scenario and Fish scenario, that is replaced with electricity and heat from EnergyDF and EnergyAD respectively.

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**Table B.1**

Datasets representing the share of energy source used to produce current, fossil, and future energy mix. Net electricity distribution was sourced from Energy-Charts (2020) on 18 December 2020.

Data generator	Dataset name	Location	%	Used to model
<b>(1) Current electricity mix</b>				
Treyer, K.	Electricity production, lignite	DE	16.9	Electricity mix
Treyer, K.	Electricity production, nuclear, pressure water reactor	DE	12.4	Electricity mix
System	Electricity production, natural gas, 10MW	DE	12.1	Electricity mix
Treyer, K.	Electricity production, wind, >3MW turbine, onshore	DE	26.6	Electricity mix
Treyer, K.	Electricity production, photovoltaic, 570kWp open ground installation, multi-Si	DE	10.7	Electricity mix
Treyer, K.	Heat and power co-generation, biogas, gas engine	DE	9.4	Electricity mix
Treyer, K.	Electricity production, hard coal	DE	7.3	Electricity mix
Treyer, K.	Electricity production, hydro, pumped storage	DE	3.9	Electricity mix
System	Electricity, high voltage, production mix	DE	0.7	Electricity mix
<b>(1) Fossil electricity mix</b>				
Treyer, K.	Electricity production, lignite	DE	33	Electricity mix
System	Electricity production, natural gas, 10MW	DE	33	Electricity mix
Treyer, K.	Electricity production, hard coal	DE	33	Electricity mix
<b>(1) Future electricity mix</b>				
Treyer, K.	Electricity production, wind, >3MW turbine, onshore	DE	45	Electricity mix
Treyer, K.	Electricity production, photovoltaic, 570kWp open ground installation, multi-Si	DE	45	Electricity mix
Treyer, K.	Heat and power co-generation, biogas, gas engine	DE	10	Electricity mix

## Appendix C

**Table C.1**

Environmental impact for each process in the *Algae scenario* and the *Fish scenario*, expressed per 1 ton DHA. GWP = Global warming [kg CO<sub>2</sub> eq], SOD = Stratospheric ozone depletion [kg CFC11 eq], IR = Ionizing radiation [kBq Co-60 eq], OF<sub>HH</sub> = Ozone formation Human health [kg NO<sub>x</sub> eq], FPMF = Fine particulate matter formation [kg PM<sub>2.5</sub> eq], OF<sub>TE</sub> = Ozone formation - Terrestrial ecosystems [kg NO<sub>x</sub> eq], TA = Terrestrial acidification [kg SO<sub>2</sub> eq], FE = Freshwater eutrophication [kg Peq], ME = Marine eutrophication [kg N eq], TETox = Terrestrial ecotoxicity [kg 1,4-DCB], FETox = Freshwater ecotoxicity [kg 1,4-DCB], METox = Marine ecotoxicity [kg 1,4-DCB], HCTox = Human carcinogenic toxicity [kg 1,4-DCB], HNCTox = Human non-carcinogenic toxicity [kg 1,4-DCB], LU = Land use [m<sup>2</sup>a crop eq], MRS = Mineral resource scarcity [kg Cu eq], FRS = Fossil resource scarcity [kg oil eq], WC = Water consumption [m<sup>3</sup>].

	GWP	SOD	IR	OF <sub>HH</sub>	FPMF	OF <sub>TE</sub>	TA	FE	ME	TETox	FETox	METox	HCTox	HNCTox	LU	MRS	FRS	WC
<b>Algae oil</b>																		
Pre-cultivation	$3.4 \times 10^1$	$1.5 \times 10^{-4}$	$2.1 \times 10^0$	$8.1 \times 10^{-2}$	$5.4 \times 10^{-2}$	$8.2 \times 10^{-2}$	$1.8 \times 10^{-1}$	$1.4 \times 10^{-2}$	$4.1 \times 10^{-2}$	$1.1 \times 10^2$	$1.0 \times 10^0$	$1.5 \times 10^0$	$1.1 \times 10^0$	$3.9 \times 10^1$	$2.0 \times 10^1$	$1.0 \times 10^{-1}$	$7.7 \times 10^0$	$9.8 \times 10^{-1}$
Pre-culture	$1.6 \times 10^3$	$2.2 \times 10^{-3}$	$4.7 \times 10^1$	$5.7 \times 10^0$	$2.2 \times 10^0$	$5.8 \times 10^0$	$5.7 \times 10^0$	$3.0 \times 10^{-1}$	$3.2 \times 10^{-1}$	$1.5 \times 10^4$	$4.4 \times 10^1$	$6.7 \times 10^1$	$5.0 \times 10^1$	$1.4 \times 10^3$	$2.2 \times 10^2$	$5.5 \times 10^0$	$4.7 \times 10^2$	$3.3 \times 10^1$
Cultivation & harvest	$4.2 \times 10^3$	$2.8 \times 10^{-3}$	$5.9 \times 10^2$	$4.0 \times 10^0$	$4.4 \times 10^0$	$4.0 \times 10^0$	$2.7 \times 10^1$	$4.1 \times 10^0$	$3.7 \times 10^{-1}$	$4.1 \times 10^3$	$2.1 \times 10^2$	$2.7 \times 10^2$	$2.1 \times 10^2$	$3.5 \times 10^3$	$4.4 \times 10^1$	$4.4 \times 10^0$	$8.0 \times 10^2$	$-4.4 \times 10^0$
Oil separation	$2.6 \times 10^3$	$2.3 \times 10^{-3}$	$4.9 \times 10^2$	$3.3 \times 10^0$	$3.7 \times 10^0$	$3.3 \times 10^0$	$2.2 \times 10^1$	$3.4 \times 10^0$	$2.2 \times 10^{-1}$	$3.5 \times 10^3$	$1.7 \times 10^2$	$2.3 \times 10^2$	$1.8 \times 10^2$	$2.9 \times 10^3$	$3.8 \times 10^1$	$3.8 \times 10^0$	$6.7 \times 10^2$	$9.9 \times 10^0$
Transport to Norway	$9.9 \times 10^2$	$4.3 \times 10^{-4}$	$1.9 \times 10^1$	$4.1 \times 10^0$	$1.2 \times 10^0$	$4.2 \times 10^0$	$2.9 \times 10^0$	$9.1 \times 10^{-2}$	$6.9 \times 10^{-3}$	$1.1 \times 10^4$	$1.8 \times 10^1$	$3.0 \times 10^1$	$2.4 \times 10^1$	$6.7 \times 10^2$	$3.5 \times 10^1$	$2.3 \times 10^0$	$3.4 \times 10^2$	$2.8 \times 10^0$
<b>Energy<sub>DF</sub></b>																		
Pre-treatment	$1.6 \times 10^4$	$1.2 \times 10^{-2}$	$9.8 \times 10^2$	$7.3 \times 10^1$	$2.3 \times 10^1$	$7.7 \times 10^1$	$7.5 \times 10^1$	$7.5 \times 10^0$	$5.0 \times 10^{-1}$	$4.5 \times 10^4$	$1.5 \times 10^3$	$2.0 \times 10^3$	$7.7 \times 10^2$	$3.1 \times 10^4$	$9.1 \times 10^2$	$4.8 \times 10^1$	$4.1 \times 10^3$	$4.5 \times 10^1$
Dark fermentation	$1.2 \times 10^3$	$1.0 \times 10^{-3}$	$2.1 \times 10^2$	$1.9 \times 10^0$	$1.9 \times 10^0$	$1.9 \times 10^0$	$9.4 \times 10^0$	$1.4 \times 10^0$	$9.1 \times 10^{-2}$	$3.1 \times 10^3$	$8.1 \times 10^1$	$1.1 \times 10^2$	$1.1 \times 10^2$	$1.6 \times 10^3$	$2.2 \times 10^2$	$5.7 \times 10^0$	$3.0 \times 10^2$	$1.3 \times 10^2$
VFA separation	$4.2 \times 10^2$	$3.7 \times 10^{-4}$	$7.8 \times 10^1$	$5.4 \times 10^{-1}$	$6.1 \times 10^{-1}$	$5.4 \times 10^{-1}$	$3.6 \times 10^0$	$5.5 \times 10^{-1}$	$3.5 \times 10^{-2}$	$6.3 \times 10^2$	$2.8 \times 10^1$	$3.7 \times 10^1$	$2.8 \times 10^1$	$4.8 \times 10^2$	$6.6 \times 10^0$	$7.3 \times 10^{-1}$	$1.1 \times 10^2$	$1.6 \times 10^0$
Anaerobic digestion	$2.3 \times 10^3$	$1.2 \times 10^{-3}$	$1.0 \times 10^2$	$8.6 \times 10^0$	$3.8 \times 10^0$	$8.8 \times 10^0$	$8.5 \times 10^0$	$7.7 \times 10^{-1}$	$4.8 \times 10^{-2}$	$2.4 \times 10^4$	$1.0 \times 10^2$	$1.5 \times 10^2$	$1.8 \times 10^2$	$3.2 \times 10^3$	$1.1 \times 10^3$	$2.4 \times 10^1$	$7.0 \times 10^2$	$1.6 \times 10^1$
Energy production	$3.0 \times 10^4$	$2.6 \times 10^{-1}$	$2.6 \times 10^2$	$2.4 \times 10^1$	$4.4 \times 10^2$	$2.4 \times 10^1$	$3.5 \times 10^3$	$3.2 \times 10^0$	$2.0 \times 10^{-1}$	$1.5 \times 10^4$	$2.1 \times 10^2$	$2.9 \times 10^2$	$3.2 \times 10^2$	$5.6 \times 10^3$	$5.2 \times 10^2$	$2.7 \times 10^1$	$1.6 \times 10^3$	$2.2 \times 10^1$
Avoided energy	$-1.1 \times 10^5$	$-4.2 \times 10^{-2}$	$-5.2 \times 10^2$	$-1.5 \times 10^2$	$-5.1 \times 10^1$	$-1.5 \times 10^2$	$-1.5 \times 10^2$	$-1.2 \times 10^2$	$-7.1 \times 10^0$	$-1.8 \times 10^4$	$-2.9 \times 10^3$	$-4.0 \times 10^3$	$-5.6 \times 10^3$	$-8.3 \times 10^4$	$-3.8 \times 10^2$	$-2.7 \times 10^1$	$-3.2 \times 10^4$	$-2.0 \times 10^2$
<b>Fish oil</b>																		
Fishing	$1.3 \times 10^4$	$3.2 \times 10^{-3}$	$1.5 \times 10^2$	$2.8 \times 10^2$	$9.1 \times 10^1$	$2.8 \times 10^2$	$2.9 \times 10^2$	$2.3 \times 10^{-1}$	$3.0 \times 10^{-2}$	$7.7 \times 10^3$	$1.8 \times 10^1$	$1.2 \times 10^3$	$5.7 \times 10^1$	$7.0 \times 10^2$	$2.3 \times 10^1$	$3.4 \times 10^0$	$4.3 \times 10^3$	$2.1 \times 10^1$
Reduction fishery	$2.0 \times 10^4$	$4.0 \times 10^{-3}$	$1.7 \times 10^2$	$1.2 \times 10^2$	$5.9 \times 10^1$	$1.2 \times 10^2$	$1.7 \times 10^2$	$4.8 \times 10^0$	$3.3 \times 10^{-1}$	$1.8 \times 10^4$	$2.4 \times 10^2$	$7.6 \times 10^2$	$3.9 \times 10^2$	$7.6 \times 10^3$	$5.6 \times 10^2$	$1.0 \times 10^1$	$4.4 \times 10^3$	$1.6 \times 10^2$
Transport to Norway	$1.1 \times 10^4$	$5.5 \times 10^{-3}$	$2.2 \times 10^2$	$6.6 \times 10^1$	$2.0 \times 10^1$	$6.7 \times 10^1$	$5.0 \times 10^1$	$1.1 \times 10^0$	$8.5 \times 10^{-2}$	$1.1 \times 10^5$	$2.0 \times 10^2$	$3.3 \times 10^2$	$2.8 \times 10^2$	$6.9 \times 10^3$	$3.4 \times 10^2$	$2.5 \times 10^1$	$3.9 \times 10^3$	$3.1 \times 10^1$
<b>Energy<sub>AD</sub></b>																		
Pre-treatment	$1.6 \times 10^4$	$1.2 \times 10^{-2}$	$9.8 \times 10^2$	$7.3 \times 10^1$	$2.3 \times 10^1$	$7.7 \times 10^1$	$7.5 \times 10^1$	$7.5 \times 10^0$	$5.0 \times 10^{-1}$	$4.5 \times 10^4$	$1.5 \times 10^3$	$2.0 \times 10^3$	$7.7 \times 10^2$	$3.1 \times 10^4$	$9.1 \times 10^2$	$4.8 \times 10^1$	$4.1 \times 10^3$	$4.5 \times 10^1$
Anaerobic digestion	$3.4 \times 10^3$	$2.2 \times 10^{-3}$	$3.0 \times 10^2$	$1.0 \times 10^1$	$5.4 \times 10^0$	$1.0 \times 10^1$	$1.7 \times 10^1$	$2.1 \times 10^0$	$1.3 \times 10^{-1}$	$2.6 \times 10^4$	$1.8 \times 10^2$	$2.5 \times 10^2$	$2.7 \times 10^2$	$4.5 \times 10^3$	$1.2 \times 10^3$	$2.7 \times 10^1$	$9.7 \times 10^2$	$1.4 \times 10^2$
Energy production	$3.0 \times 10^4$	$2.6 \times 10^{-1}$	$2.6 \times 10^2$	$2.4 \times 10^1$	$4.4 \times 10^2$	$2.4 \times 10^1$	$3.5 \times 10^3$	$3.2 \times 10^0$	$2.0 \times 10^{-1}$	$1.5 \times 10^4$	$2.1 \times 10^2$	$2.9 \times 10^2$	$3.2 \times 10^2$	$5.6 \times 10^3$	$5.2 \times 10^2$	$2.7 \times 10^1$	$1.6 \times 10^3$	$2.2 \times 10^1$
Avoided energy	$-1.1 \times 10^5$	$-4.2 \times 10^{-2}$	$-5.1 \times 10^2$	$-1.5 \times 10^2$	$-5.0 \times 10^1$	$-1.5 \times 10^2$	$-1.5 \times 10^2$	$-1.2 \times 10^2$	$-7.1 \times 10^0$	$-1.7 \times 10^4$	$-2.9 \times 10^3$	$-4.0 \times 10^3$	$-5.6 \times 10^3$	$-8.3 \times 10^4$	$-3.8 \times 10^2$	$-2.6 \times 10^1$	$-3.1 \times 10^4$	$-2.0 \times 10^2$

**Table C.2**

Ecosystem damage [species.yr] for each process in the *Algae scenario* and the *Fish scenario*, expressed per 1 ton DHA.  $GWP_{TE}$  = Global warming - Terrestrial ecosystems,  $GWP_{FE}$  = Global warming - Freshwater ecosystems,  $OF_{TE}$  = Ozone formation - Terrestrial ecosystems, TA = Terrestrial acidification, FE = Freshwater eutrophication, ME = Marine eutrophication, TETox = Terrestrial ecotoxicity, FETox = Freshwater ecotoxicity, METox = Marine ecotoxicity, LU = Land use,  $WC_{TE}$  = Water consumption - Terrestrial ecosystems,  $WC_{AE}$  = Water consumption - Aquatic ecosystems.

	$GWP_{TE}$	$GWP_{FE}$	$OF_{TE}$	TA	FE	ME	TETox	FETox	METox	LU	$WC_{TE}$	$WC_{AE}$
<b>Algae oil</b>												
Pre-cultivation	$9.5 \times 10^{-8}$	$2.6 \times 10^{-12}$	$1.1 \times 10^{-8}$	$3.8 \times 10^{-8}$	$9.2 \times 10^{-9}$	$7.0 \times 10^{-11}$	$1.3 \times 10^{-9}$	$7.1 \times 10^{-10}$	$1.5 \times 10^{-10}$	$1.8 \times 10^{-7}$	$9.7 \times 10^{-9}$	$4.5 \times 10^{-13}$
Starting culture	$4.5 \times 10^{-6}$	$1.2 \times 10^{-10}$	$7.5 \times 10^{-7}$	$1.2 \times 10^{-6}$	$2.0 \times 10^{-7}$	$5.5 \times 10^{-10}$	$1.7 \times 10^{-7}$	$3.0 \times 10^{-8}$	$7.0 \times 10^{-9}$	$2.0 \times 10^{-6}$	$4.0 \times 10^{-7}$	$2.9 \times 10^{-11}$
Cultivation & harvest	$1.2 \times 10^{-5}$	$3.2 \times 10^{-10}$	$5.2 \times 10^{-7}$	$5.7 \times 10^{-6}$	$2.7 \times 10^{-6}$	$6.2 \times 10^{-10}$	$4.7 \times 10^{-8}$	$1.4 \times 10^{-7}$	$2.9 \times 10^{-8}$	$3.9 \times 10^{-7}$	$-9.0 \times 10^{-8}$	-
Oil separation	$7.3 \times 10^{-6}$	$2.0 \times 10^{-10}$	$4.3 \times 10^{-7}$	$4.7 \times 10^{-6}$	$2.3 \times 10^{-6}$	$3.7 \times 10^{-10}$	$4.0 \times 10^{-8}$	$1.2 \times 10^{-7}$	$2.4 \times 10^{-8}$	$3.4 \times 10^{-7}$	$1.1 \times 10^{-7}$	$4.9 \times 10^{-12}$
Transport to Norway	$2.8 \times 10^{-6}$	$7.5 \times 10^{-11}$	$5.4 \times 10^{-7}$	$6.1 \times 10^{-7}$	$6.1 \times 10^{-8}$	$1.2 \times 10^{-11}$	$1.2 \times 10^{-7}$	$1.3 \times 10^{-8}$	$3.2 \times 10^{-9}$	$3.1 \times 10^{-7}$	$3.2 \times 10^{-8}$	$1.6 \times 10^{-12}$
<b>Energy<sub>DF</sub></b>												
Pre-treatment	$4.5 \times 10^{-5}$	$1.2 \times 10^{-9}$	$9.9 \times 10^{-6}$	$1.6 \times 10^{-5}$	$5.0 \times 10^{-6}$	$8.5 \times 10^{-10}$	$5.1 \times 10^{-7}$	$1.0 \times 10^{-6}$	$2.1 \times 10^{-7}$	$8.1 \times 10^{-6}$	$3.8 \times 10^{-7}$	$1.8 \times 10^{-11}$
Dark fermentation	$3.3 \times 10^{-6}$	$9.1 \times 10^{-11}$	$2.5 \times 10^{-7}$	$2.0 \times 10^{-6}$	$9.5 \times 10^{-7}$	$1.6 \times 10^{-10}$	$3.6 \times 10^{-8}$	$5.6 \times 10^{-8}$	$1.1 \times 10^{-8}$	$1.9 \times 10^{-6}$	$1.7 \times 10^{-6}$	$7.5 \times 10^{-11}$
VFA separation	$1.2 \times 10^{-6}$	$3.2 \times 10^{-11}$	$7.0 \times 10^{-8}$	$7.7 \times 10^{-7}$	$3.7 \times 10^{-7}$	$6.0 \times 10^{-11}$	$7.2 \times 10^{-9}$	$2.0 \times 10^{-8}$	$3.9 \times 10^{-9}$	$5.9 \times 10^{-8}$	$1.7 \times 10^{-8}$	$8.0 \times 10^{-13}$
Anaerobic digestion	$6.5 \times 10^{-6}$	$1.8 \times 10^{-10}$	$1.1 \times 10^{-6}$	$1.8 \times 10^{-6}$	$5.1 \times 10^{-7}$	$8.1 \times 10^{-11}$	$2.7 \times 10^{-7}$	$7.1 \times 10^{-8}$	$1.6 \times 10^{-8}$	$9.8 \times 10^{-6}$	$1.5 \times 10^{-7}$	$7.6 \times 10^{-12}$
Energy production	$8.5 \times 10^{-5}$	$2.3 \times 10^{-9}$	$3.1 \times 10^{-6}$	$7.4 \times 10^{-4}$	$2.1 \times 10^{-6}$	$3.4 \times 10^{-10}$	$1.7 \times 10^{-7}$	$1.5 \times 10^{-7}$	$3.1 \times 10^{-8}$	$4.6 \times 10^{-6}$	$1.9 \times 10^{-7}$	$1.8 \times 10^{-11}$
Avoided energy	$-3.1 \times 10^{-4}$	$-8.5 \times 10^{-9}$	$-1.9 \times 10^{-5}$	$-3.2 \times 10^{-5}$	$-7.7 \times 10^{-5}$	$-1.2 \times 10^{-8}$	$-2.0 \times 10^{-7}$	$-2.0 \times 10^{-6}$	$-4.2 \times 10^{-7}$	$-3.4 \times 10^{-6}$	$-2.5 \times 10^{-6}$	-
												$1.1 \times 10^{-10}$
<b>Fish oil</b>												
Fishing	$3.6 \times 10^{-5}$	$9.9 \times 10^{-10}$	$3.7 \times 10^{-5}$	$6.1 \times 10^{-5}$	$1.6 \times 10^{-7}$	$5.1 \times 10^{-11}$	$8.8 \times 10^{-8}$	$1.3 \times 10^{-8}$	$1.3 \times 10^{-7}$	$2.0 \times 10^{-7}$	$2.7 \times 10^{-7}$	$1.3 \times 10^{-11}$
Reduction fishery	$5.5 \times 10^{-5}$	$1.5 \times 10^{-9}$	$1.5 \times 10^{-5}$	$3.6 \times 10^{-5}$	$3.2 \times 10^{-6}$	$5.6 \times 10^{-10}$	$2.0 \times 10^{-7}$	$1.7 \times 10^{-7}$	$8.0 \times 10^{-8}$	$5.0 \times 10^{-6}$	$1.9 \times 10^{-6}$	$1.4 \times 10^{-10}$
Transport to Norway	$3.2 \times 10^{-5}$	$8.8 \times 10^{-10}$	$8.6 \times 10^{-6}$	$1.1 \times 10^{-5}$	$7.2 \times 10^{-7}$	$1.4 \times 10^{-10}$	$1.2 \times 10^{-6}$	$1.4 \times 10^{-7}$	$3.5 \times 10^{-8}$	$3.1 \times 10^{-6}$	$3.6 \times 10^{-7}$	$1.9 \times 10^{-11}$
<b>Energy<sub>AD</sub></b>												
Pre-treatment	$4.5 \times 10^{-5}$	$1.2 \times 10^{-9}$	$9.9 \times 10^{-6}$	$1.6 \times 10^{-5}$	$5.0 \times 10^{-6}$	$8.5 \times 10^{-10}$	$5.1 \times 10^{-7}$	$1.0 \times 10^{-6}$	$2.1 \times 10^{-7}$	$8.1 \times 10^{-6}$	$3.8 \times 10^{-7}$	$1.8 \times 10^{-11}$
Anaerobic digestion	$9.5 \times 10^{-6}$	$2.6 \times 10^{-10}$	$1.3 \times 10^{-6}$	$3.7 \times 10^{-6}$	$1.4 \times 10^{-6}$	$2.3 \times 10^{-10}$	$2.9 \times 10^{-7}$	$1.2 \times 10^{-7}$	$2.6 \times 10^{-8}$	$1.1 \times 10^{-5}$	$1.8 \times 10^{-6}$	$8.3 \times 10^{-11}$
Energy production	$8.5 \times 10^{-5}$	$2.3 \times 10^{-9}$	$3.1 \times 10^{-6}$	$7.4 \times 10^{-4}$	$2.1 \times 10^{-6}$	$3.4 \times 10^{-10}$	$1.7 \times 10^{-7}$	$1.5 \times 10^{-7}$	$3.1 \times 10^{-8}$	$4.6 \times 10^{-6}$	$1.9 \times 10^{-7}$	$1.8 \times 10^{-11}$
Avoided energy	$-3.1 \times 10^{-4}$	$-8.4 \times 10^{-9}$	$-1.9 \times 10^{-5}$	$-3.2 \times 10^{-5}$	$-7.7 \times 10^{-5}$	$-1.2 \times 10^{-8}$	$-2.0 \times 10^{-7}$	$-2.0 \times 10^{-6}$	$-4.2 \times 10^{-7}$	$-3.4 \times 10^{-6}$	$-2.4 \times 10^{-6}$	-
												$1.1 \times 10^{-10}$

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