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Life cycle assessment of DHA produced by microalgae using food waste

Assessing global warming, fossil energy use and effects on biodiversity

Louise Bartek

Abstract

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Biodiversity is a key component for life on Earth since it contributes to clean water, fresh air and food security. Today, fatty fish farmed in aquaculture is the main Omega 3 source consumed by humans, including the essential fatty acid *docosahexaenoic acid* (DHA). DHA origin from plants and accumulate in fish via the marine food web. Therefore, DHA in the form of fish oil is often added to fish feed used in aquaculture. This process is dependent on fossil energy and marine raw materials, which infer increased global warming, damage to ecosystem and ultimately loss of biodiversity. In order to reduce the environmental impact, the essential fatty acid could instead be derived from the marine primary producer of DHA: microalgae.

In this thesis, a life cycle approach was used to assess global warming, use of fossil fuels and Ecosystem damage when DHA is produced by the microalgae *Crypthecodinium Cohnii*. The environmental impact was modelled using SimaPro 9 and assessed with CML-IA and ReCiPe Endpoint. In this model, volatile fatty acids derived from dark fermentation of food waste was used as feedstock to the algae. The studied systems consisted of two parallel scenarios, one conventional food waste-to-biogas with DHA from fish oil and one conceptual food waste-to-DHA with DHA from algae oil. The aim was to evaluate the future potential of DHA produced from algae, by assessing and comparing environmental impact to DHA produced from Peruvian anchovy.

For every ton DHA produced by microalgae the assessed impact was -1.9E+02 tonCO_2e, -1.9 TJ and 9.7E-04 species.yr. DHA produced by microalgae using VFA from food waste was shown to mitigate global warming and reduce use of fossil fuels. The most important conclusion show that DHA from algae infer 37% lower biodiversity loss in comparison to DHA from Peruvian anchovy. Thus, DHA from microalgae could reduce dependency on marine raw material and decrease biodiversity loss.

Keywords: Life cycle assessment (LCA), docosahexaenoic acid (DHA), biodiversity loss, volatile fatty acids (VFA), global warming, sustainable development.

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Referat

Livscykelanalys för DHA producerat av mikroalger via matavfall
Louise Bartek

Biodiversitet är en nyckelkomponent för liv på jorden eftersom det bidrar till rent vatten, frisk luft och säker livsmedelsproduktion. Idag är fet fisk odlad i vattenbruk den viktigaste källan till Omega 3 som konsumeras av människor, inklusive den essentiella fettsyran *dokosahexaensyra* (DHA). Då DHA härstammar från växter och ackumuleras i fisk via den marina näringskedjan, tillsätts DHA ofta till fiskfoder i form av fiskolja. Denna process är beroende av fossil energi och marina råmaterial, som leder till ökad global uppvärmning, skadar naturliga ekosystem och orsakar förlust av biologisk mångfald. För att minska miljöpåverkan skulle den essentiella fettsyran istället kunna produceras från den marina primärproducenten av DHA: mikroalger.

I detta examensarbete användes livscykelanalys för att utvärdera miljöpåverkan, med avseende på global uppvärmning, användning av fossila bränslen och påverkan på biodiversitet, då DHA produceras av mikroalgen *Crypthecodinium Cohnii*. Flyktiga fettsyror, VFA, som bildas vid mörk fermentering av matavfall användes som råmaterial till algerna. De studerade systemen bestod av två parallella scenarier, en konventionell matavfall-till-biogas med DHA från fiskolja och en konceptuell matavfall-till-DHA med DHA från algolja. Systemet modellerades i SimaPro 9 och miljöpåverkan beräknades med CML-IA och ReCiPe Endpoint. Syftet var att utvärdera DHA som produceras från alger, genom att beräkna miljöpåverkan och jämföra med DHA producerad från peruansk ansjovis.

För varje ton DHA producerat av mikroalger var påverkan -1.9E+02 tonCO_2e, -1.9 TJ och 9.7E-04 arter per år. DHA producerad av mikroalger där VFA från matavfall använts som näring, visade sig minska den globala uppvärmningen, reducera användningen av fossila bränslen och innebar 37% lägre förlust av biologisk mångfald jämfört med DHA producerad från peruansk ansjovis. Denna studie visade därmed att DHA från mikroalger kunde minska beroendet av marina råmaterial och minska förlusten av biologisk mångfald.

Nyckelord: Livscykelanalys (LCA), dokosahexaensyra (DHA), biodiversitet, flyktiga fettsyror (VFA), global uppvärmning, hållbar utveckling.

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Preface

This thesis comprises 30 higher education credits and conclude my studies at the Civil Engineering Program in Environmental and Water Engineering at Uppsala University (UU) and Swedish University of Agricultural Sciences (SLU). Supervisor was Ingrid Strid and subject reader was Mattias Eriksson, researchers at the Department of Energy and Technology at SLU.

First and foremost, I would like to thank my supervisor Ingrid and subject reader Mattias for this opportunity and for valuable feedback and guidance during my master thesis. I have had the chance to hone my problem solving skills and intuitive ability, which I believe have made me a better engineer. Furthermore, I would like to express gratitude to Åke Nordberg who always took the time to answer my questions and assist with calculations. A special thanks is also dedicated to Luboš for helping me to unlock closed doors and to Alena for invaluable feedback. Since day one at Uppsala University I have had the pleasure of getting to know people who have gilded my days and contributed to my eagerness to make a difference in the world. Over endless cups of coffee we have learned that nothing is impossible. I am grateful for my family and friends who have encouraged me all the way and for Florence who always make me smile. And finally to Hans; your support truly means the world to me.

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Populärvetenskaplig sammanfattning

Sedan den industriella revolutionen har människan varit beroende av fossil energi för att driva den tekniska och sociala utveckling som lett till dagens moderna samhälle. Dessvärre har utvecklingen även orsakat allvarliga samhällsproblem i form av utarmade naturresurser, föroreningar och klimatförändringar. Vattenbruk, som avser all odling i vattenmiljöer, har utvecklats för att försörja den globala befolkningsökningen med odlad fisk och den essentiella fettsyran Omega 3. Länge ansågs fet fisk vara den bästa källan till Omega 3, i synnerhet *dokosahexaensyra* (DHA). Utan DHA skulle få organismer på jorden överleva, då den är nödvändig för god hälsa och hjärnans normala utveckling.

I marina ekosystem är växtliknande mikroalger primärproducenter av DHA, medan fisk får i sig DHA genom att äta dessa alger. När fisk odlas i vattenbruk tillsätts DHA vanligtvis i fiskfodret genom fiskolja från viltfångad fisk. Idag odlas 80 miljoner ton fisk i vattenbruk, vilket innebär att enorma mängder vildfångad fisk tas upp ur världshaven för att producera DHA till fiskfoder. Denna process kräver även stora mängder fossil energi, landyta och naturresurser. Trots att fiskodling till en början ansågs lösa den globala utmaningen med överfiske, bidrar det istället till två av de mest akuta hoten mot liv på jorden: global uppvärmning och förlust av biologisk mångfald.

Biologisk mångfald och välfungerande ekosystem är en förutsättning för liv på jorden, då det bidrar till rent vatten, frisk luft och en långsiktig matproduktion. För att främja en hållbar utveckling, där jordens resurser används på ett cirkulärt sätt och viktiga ekosystem bevaras, krävs nya produktionsmetoder med lägre miljöpåverkan. Ett lovande forskningsområde är tekniker som utvinner DHA-rika oljor direkt från marina primärproducenter av DHA, nämligen mikroalger. Flera studier har visat att mikroalgerna dessutom kan odlas med hjälp av flyktiga fettsyror (VFA) som bildas vid nedbrytning av organiskt matavfall. I teorin skulle DHA kunna produceras i stor skala av odlade mikroalger som matas med VFA från matavfall, vilket skulle minska behovet av viltfångad fisk inom fiskodling samt bidra till en cirkulär matavfallshantering.

Livscykelanalys (LCA) är en metod för att bedöma miljöpåverkan för en produkt eller process, genom att mängden ingående resurser och emissioner från utvinning av råvara till avfallshantering identifieras. I detta examensarbete används LCA som ett verktyg för att bedöma miljöpåverkan för en process där DHA produceras av marina mikroalger som matas med VFA från matavfall. För att bedöma miljöpåverkan från DHA producerad av mikroalger har en modell av systemet skapats i modelleringsverktyget SimaPro 9, där exempelvis mängden matavfall, alger och energi beskrevs med data från databasen Ecoinvent 3.5. Mikroalgen *Crypthecodinium cohnii* är en marin primärproducent av DHA som kan odlas i mörker genom att tillsätta näring. Den huvudsakliga näringen var VFA som utvinns från så kallad *mörk fermentering* av matavfall i en biogasreaktor. Då endast en liten del VFA bildas i fermenteringsprocessen, antogs resten av matavfallet återanvändas för att producera biogas för elektricitet och värmeanvändning. Mängden förlorad biogas på grund av att VFA extraherats beräknades och inkluderades i modellen. För att utvärdera DHA producerad från alger skapades även en modell för fiskolja, som idag är den komersiella DHA källan. Miljöpåverkan bedömdes med avseende på global uppvärmning, fossil energianvändning och påverkan på biologisk mångfald.

Studiens resultat visar att DHA som producerats av odlade mikroalger matade mad VFA från matavfall, har en lägre miljöpåverkan i jämförelse med fiskolja per ton producerad DHA. I dagsläget gör biogasproduktionen att användandet av fossil energi minskar, då biogasen kan avnändas för att ersätta fossila energikällor. Resultatet i denna studie visar att när användandet av fossil energi minskar inom produktionsprocessen av algolja och fiskolja, reduceras den totala miljöpåverkan för respektive process. Resultatet visar även att global uppvärmning och fossil energianvändning minskar när DHA produceras från algolja, och ökar om DHA produceras från fiskolja. Trots att etablerade LCA metoder för biodiversitet i dagsläget underskattar miljöpåverkan, är en av studiens viktigaste slutsatser att DHA från algolja leder till lägre förlust av biologisk mångfald i jämförelse med fiskolja.

Contents

Definition of concepts

Abiotic resource - Natural resources that are nonliving, such as water, land and air. Abiotic depletion refers to the depletion of nonliving resources, such as fossil fuels.

Algae oil - Is produced by pressing marine microalgae to separate oil from solids. Depending on the microalgae species, algae oil have a specific nutritional composition and can be used within food or aquaculture production.

Anaerobic digestion - Refers to the degradation process of biodegradable materials by microorganisms in absence of oxygen. Anaerobic digestion (AD) can be used to produce biogas from food waste.

Aquaculture - A term that includes all farming activities in water environments, such as breeding and harvesting fish or algae.

Biodiversity - Is defined as the variability among all living organisms, including plants and animals in marine, aquatic and terrestrial ecosystems. Biodiversity is the foundation for essential ecosystem services vital for life on Earth.

Biogas - Is a renewable source of energy produced from biomass, such as food waste. It consist of about 60% methane and 40% carbon dioxide. Biogas can be used to produce electricity and heat via combustion, or upgraded to pure methane used as fuel.

Biotic resource - Natural resources that are living, such as plants, animals and marine organisms.

Dark fermentation - Refers to the fermentation of organic substance by microorganisms in absence of light. Dark fermentation (DF) convert organic substance to hydrogen, and can also be used to produce VFA from food waste.

DHA - Docosahexaenoic acid (DHA) is one of two essential fatty acids commonly referred to as Omega 3. DHA is widely used in food and supplement production due to its health benefits.

Food waste - Comprises wasted food appropriate for human consumption. Commonly, both vegetable and animal based waste are included.

LCA - Life cycle assessment is a systematic method to develop a holistic picture of total environmental impact caused by a process or a product.

Microalgae - A plant-like organism fundamental for marine ecosystems as they supply higher trophic levels with nutrients and essential fatty acids.

Ton - In this thesis, ton refer to metric ton i.e. 1000 kg.

VFA - Volatile Fatty Acids (VFA) comprise organic short-chain fatty acids produced via anaerobic digestion or dark fermentation of organic matter.

1 Introduction

Since the industrial revolution, humanity has used fossil fuels and natural resources to power a technical and social development. Alongside, human impact has led to increased greenhouse gas emissions, depleted natural resources and damage to vital ecosystems (United Nations 2019; Harfoot et al 2018). The fastest growing food producing sector today is aquaculture, developed to support an increasing global population with nutritious food and Omega 3 fatty acids (FAO 2018).

In the beginning, aquaculture was considered a solution to decreased biodiversity and diminished ecosystems, as a result of overfishing. By farming high value fish, such as salmon, the aim was to maintain sustainable wild fish populations and preserve marine biodiversity (European commission 2019a). Meanwhile, the increased global demand for fish rich in essential fatty acids could be satisfied. However, modern aquacultures are often described as industrial-sized farms under water, relying on large amounts of marine raw materials, energy and antibiotics (Crawford & Macleod 2009). When the global demand for fish increase, natural resources and ecosystems are depleted. Instead of solving the problem of overfishing, aquaculture created new ones (WWF 2012; FAO 2019).

Until recently, fatty fish such as salmon was considered the best source of essential fatty acids vital for human health, such as *docosahexaenoic acid* (DHA) found in *Omega 3*. However, DHA accumulates via the food web in top predators consumed by humans (Colombo et al 2019). In aquatic ecosystems, DHA is naturally produced by plant-like microalgae while fish obtain DHA by eating algae (Shahidi & Ambigaipalan 2019). The high DHA content in farmed salmon is today accomplished by adding DHA rich oils to the salmon feed (Toppe 2013), a process that contribute to two of the most urgent threats to life on Earth: global warming and loss of biodiversity (United Nations 2019; Center for Biological Diversity 2020).

In order to support sustainable development, new production methods with lower environmental impact are in high demand. One promising area of research are techniques which extract DHA rich oils directly from cultivated microalgae, thus providing an alternative way to produce the essential fatty acids from the primary producer. Alongside, research has shown that microalgae farmed in aquaculture can grow on fatty acids derived from food waste (Chalima et al 2020), which thereby could enable a combination of aquaculture production with a valorization method for food waste (Tampio et al 2018; Chalima et al 2017). Today, biogas used for renewable energy production is an established valorization method for food waste, but a similar process can also be used to produce VFA (Paritosh et al 2017; Strazzera et al 2018). In theory, DHA produced from microalgae fed VFA derived from food waste could reduce environmental impact by decreasing the demand for marine raw materials and mitigate global warming. A framework which has become increasingly used to quantify environmental impact for a product or process is *life cycle assessment* (LCA). By using an LCA approach, resource demanding flows and environmental impact related to DHA produced from algae oil can be assessed (Woods et al 2016). The impact from algae oil can thereafter be evaluated in comparison to fish oil, to identify whether the suggested approach could reduce environmental impact.

1.1 Background

The project AVARE, short for *Adding value in resource effective food systems*, is a transnational collaboration with actors from Sweden, Germany, Norway and Finland founded by the *SUSFOOD2 ERA-Net* (2020). The main objective is to promote sustainable development within the food sector by increasing resource efficiency, adding value to food waste and developing alternative uses for food waste.

As a part of the AVARE project, a small-scale waste-to-value technique was developed where DHA can be produced from food waste. The technique utilize the microalgae *Crypthecodinium cohnii* (C.Cohnii) as primary producer of DHA, where *Volatile fatty acids* (VFA) derived from *dark fermentation* of food waste serve as a carbon feedstock to the algae (Technical University of Berlin 2019). When microalgae increase in biomass they accumulate a DHA rich algae oil that could be used within food production. The algae oil could potentially substitute fish oil traditionally used in fish feed and thereby decrease impact on biodiversity.

1.2 Study objective and research questions

This thesis will assess the environmental impact of DHA produced in a large scale from food waste, using VFA derived from dark fermentation as feed to C.Cohnii algae. The suggested approach will compared with the commercial DHA source, fish oil, with respect to global warming, fossil energy use and effects on biodiversity. The long-term goal is to support sustainable development, by assessing waste-to-value techniques. To achieve this, the following research questions were posed:

- What is the environmental impact per ton DHA produced by microalgae when using VFA derived from food waste as feedstock?
- What is the difference in environmental impact between algae oil and fish oil with respect to global warming, fossil energy use and effects on biodiversity?

1.3 Research delimitations

In this thesis, established LCA methods available for commercial use were used. Although LCA methods are constantly evolving, no method is yet able to link all direct and indirect effects on biodiversity to a common unit (Winter et al 2017; Asselin et al 2020). The ReCiPe Endpoint (H) was used in this thesis to assess indirect effects on biodiversity. This delimitation ensure compatibility with established methods, while the suggested research methods are considered in the discussion.

2 Theory

The following sections will introduce the reader to different aspects of sustainable development, with emphasis on biodiversity and waste-to-value techniques. Alongside, important factors affecting DHA produced from microalgae and fish oil will be addressed. Current technical requirements and conditions for production are included and supported by previous research. Lastly, the LCA structure and methodology is presented in order to describe the basis of this thesis.

2.1 Sustainable development

The principle of sustainable development is to promote actions that maintain nature's ability to provide resources and ecosystem services, while ensuring that basic human rights are satisfied (World Commission on Environment and Development 1987). It refers to development that meets the needs of today without compromising the needs of future generations. The Sustainable Development Goals (SDG) developed by the United Nations (2019) is a part of Agenda 2030, and consists of 17 global goals designed to achieve a more sustainable future. The goals range from *climate change actions* to *cleaner energy production* and *conservation of biodiversity*. The common objective is to provide a plan of action to encourage people and policy makers to act in favor of sustainable development.

2.1.1 Global warming and biodiversity

The primary cause of climate change is burning of fossil fuels that increase levels of greenhouse gases (GHG) in the atmosphere. Some of the most important GHG are water vapor (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and ozone (O_3) (SMHI 2020). In short, when incoming short-wave radiation from the Sun reach the Earth, energy is absorbed by the surface and the atmosphere, while some is reflected back to space. The Earth emit long-wave radiation and when the atmospheric level of GHG increase, more long-wave radiation is absorbed and emitted by the GHG molecules. This cause an increased surface temperature (Bernes 2016). Global warming refers to the long-term temperature increase caused by human activities observed since the pre-industrial era (NASA 2020). The environmental impacts of global warming and climate change are extensive and range from increased frequency of weather extremes and damage to natural ecosystems (Holmgren 2019; IPCC 2018). As the name implies, the consequences occur at a global scale, thus affecting all plants, animals and humans on Earth (Bernes 2016).

One key aspect of sustainable development is to promote conservation of biodiversity in marine, coastal and terrestrial ecosystems. Natural ecosystems have a unique ability to purify air and water, provide vital natural resources and a wide range of essential ecosystem services, while counteracting climate change and the spread of diseases (FAO 2019). Ultimately, healthy ecosystems with a rich biodiversity are fundamental to life on earth (Center for Biological Diversity 2020).

Today, biodiversity is declining at an alarming rate and around 1 million animal and plant species are currently threatened with extinction (Center for Biological Diversity 2020). The damage occurring to marine and terrestial ecosystems is mainly caused by human activities, such as greenhouse gas emissions and land-use (United Nations 2019; Woods et al 2016). The impact has been related to five so-called *drivers for biodiversity loss*, identified by Millenium Ecosystem Assessment (2005) namely: *climate change*, *nutrient pollution*, *change in habitat*, *overexploatation* and *invasive species*. These drivers serve as indicators for Ecosystem damage and provide a measurable link between human actions and ecosystem impact. According to the Convention on Biological Diversity (2018), climate change is estimated to be the most significant driver of biodiversity loss by 2100. Additionally, overexploatation and invasive species have been identified as main drivers for marine biodiversity loss (Woods et al 2016; Emanuelsson et al 2014).

2.1.2 Fossil fuels and pollutants

The extraction and use of fossil fuels impact biodiversity both indirectly through climate change, and directly via increased pollution and land use (FAO 2019; Harfoot et al 2018). Transport related emissions cause about a quarter of the GHG emissions in Europe, hence contributing to global warming and increased pollution (European Commission 2016). Sustainable development promote reduction of fossil fuels used for energy and transport, in favor of renewable energy sources such as wind, photovoltaics and biomass. Alongside, WWF (2012) has established a vision of 100% renewable energy by 2050 and part of the SDG climate action is to develop low- and zero-emission vehicles. The *European Emission standard* (Euro standard) refers to the acceptable limit of exhaust emissions for new vehicles sold in the European Union, where a higher Euro standard infer stricter emission limits. In January 2013, the emission standard *Euro VI* became mandatory for heavy duty vehicles such as lorries (European commission 2019b).

Use of fossil energy, especially burning of fossil fuels, emits pollutants harmful to the environment and human health (NASA 2020). For instance, fossil fuels emit carbon dioxide, sulfur dioxide (SO_2) and nitrogen oxide (NO_X). Carbon dioxide increase concentration of GHG in the atmosphere, which cause global warming (Boberg 2020; Bernes 2016). Sulfur dioxide cause acidification by emitting sulfur to the air, that via precipitation accelerate acidification in water and soil (Valinia 2019). Nitrogen oxide contribute to eutrophication, acidification and ozone formation, by causing excessive levels of nitrogen in soil, water and air. Some of the main pollution sources are road and sea transport, industry processing and energy production (Ek 2019). According to Intergovernmental Panel on Climate Change (IPCC 2018), decreased dependency on fossil energy is important to promote sustainable development.

2.1.3 Omega-3 fatty acids

Another important aspect of sustainable development is to ensure that an increasing population has access to nutritiously dense food. Many so-called essential nutrients, such as vitamins and fatty acids, are vital for multiple life support functions but can only be obtained through diet. Some of the most important essential fatty acids are *Alpha linolenic acid (ALA)*, *Docosahexaenoic acid (DHA)* and *Eicosapentaenoic acid (EPA)* (Shahidi & Ambigaipalan 2019).

ALA is an essential fatty acid found in plants, from which the polysaturated fatty acids EPA and DHA are synthesized through digestion (Shahidi & Ambigaipalan 2019). EPA regulates inflammation and promotes cell function, while DHA plays a multi-functional role in preventing diseases and promoting brain health (Winwood 2013). Since DHA cannot be produced by animals, a sufficient intake must be obtained through diet. Therefore, DHA is often added in food production to enhance nutrition levels (Silva et al 2019). This is often accomplished by adding fish oil rich in DHA to animal feed to increase nutrition in dairy, meat and fish consumed by humans (Toppe 2013; Ganesan et al 2014). In a recent study by Colombo et al (2019) its concluded that global warming and increased water temperatures can reduce DHA synthesis at the base of aquatic food chains with 58% until 2100. This would reduce the amount of DHA available to humans and animals. According to Holmgren (2019) one of the most tangible consequences of climate change and loss of biodiversity for humans, is likely to be an altered food security due to increased temperatures and change in natural ecosystem functions.

2.2 Waste-to-value

The idea behind a waste-to-value approach is to recover energy and nutrients and re-use them, hence creating a circular loop where no resources are wasted. Today, food waste accounts for a large portion of human's negative environmental impact, since about one third of food produced each year is wasted (FAO 2017). This equals to 1.3 billion ton every year (FAO 2013) and approximately 8% of the total GHG emissions (European commission 2018). Simultaneously, global resources are depleted when raw materials are extracted instead of using already available resources. According to *The waste hierarchy*, waste should primarily be avoided and secondarily reused. Reusing food waste should be consistent with the most efficient use to optimized recourse recovery (EPA 2017). In order to achieve a sustainable development, where resources are re-used and existing ecosystems are maintained, multiple waste-to-value techniques are required.

Globally, some common ways of treating food waste is to generate electricity and heat via municipal incineration, re-use to produce biogas or discard to landfill (World Biogas Association 2019). Another potential solution is to re-use food waste as feed to animals, a practice that has been applied in small scale production for centuries. However, due to the risk of contamination when animal by-products are used as feed in large scale industries, a EU legislation on animal by-products was established. This entails a ban on feeding production animals with food waste (Jordbruksverket 2018).

2.2.1 Biogas

Biogas is a renewable source of energy that mainly consists of methane produced from organically degradable material. Today, biogas production is an established valorization method for complex organic waste materials, and is considered one of the most efficient ways to re-use food waste (Paritosh et al 2017). One of the main objectives with biogas production and use is to reduce fossil energy consumption and ultimately mitigate global warming. Moreover, biogas use contributes to achieving nine SDG goals, including *climate action*, *clean energy* and *life below water* (World Biogas Association 2018).

Biogas can for instance be used in combustion to generate electricity and heat, or upgraded to biomethane used as vehicle fuel (World Biogas Association 2019; IEA 2020). When simplified, the biogas process consists of three steps: *pre-treatment*, *anaerobic digestion* and *biogas production*, resulting in energy and a digestate, see Figure 1.

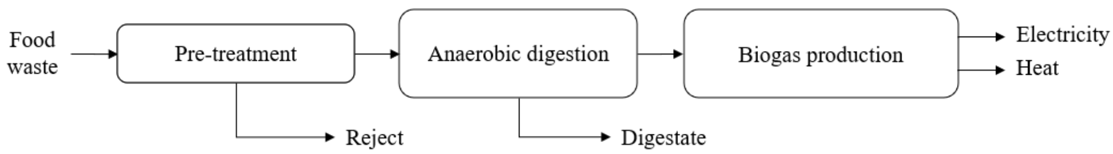


Figure 1: Simplified process for biogas production from food waste, when used to produce electricity and heat. Additional energy, water, transports and processing units are excluded in this illustration.

The *pre-treatment* begins when collected food waste is delivered to the biogas plant.

This is a necessary step for sorting out unwanted materials, such as plastic and metal. A study by Bernstad & Jansen (2011) assumed the amount of reject to be 18% of collected food waste, that mainly goes to combustion with energy recovery. The remaining slurry is pumped to a two-step *anaerobic digestion* (AD), where microorganisms convert organic matter into biogas and a digestate. The first step in AD is *hydroysis* where bacteria hydrolyses food waste into small-chain carbohydrates, amino acids and long-chain fatty acids (Paritosh et al 2017). This process enables the bacteria to begin a fermentation process consisting of *acidogenesis* and *acetogenesis*, where intermediate compounds such as volatile fatty acids (VFA), hydrogen (H_2) and carbon dioxide (CO_2) are produced (Wainaina et al 2019). According to Paritosh et al (2017) the VFA concentration in AD could rise to 20 g/L, but is in practice kept low to promote the formation of methane during *methanogenesis*. The methanogenesis is the final step where VFA and H_2 are used to produce biogas (Wainaina et al 2019). In this process, 60% biogas and 40% digestate is produced where the biogas consist of 60% methane (CH_4) and 40% CO_2 (Camacho et al 2019; World Biogas Association 2019). The *biogas production* refers to the utilization of biogas to produce energy, primarily electricity and heat by combustion using a high efficiency biogas engine. The digestate has a low market value but can be re-used as fertilizer within aquaculture (Rutz et al 2015) or agriculture since it has similar properties as organic fertilizer (Klackenberg 2019; World Biogas Association 2019).

2.2.2 Dark fermentation

Another waste-to-value valorization method that has gained a lot of scientific attention is *dark fermentation* (DF). The process comprise fermentation of organic substance in absence of light, where volatile fatty acids are produced as a by-product in H_2 production (Chalima et al 2017).

In summary, anaerobic digestion and dark fermentation both utilize anaerobic fermentation where the first two steps, hydrolysis and acidogenesis, are the same. However, the aim with AD is to produce CH_4 while the aim with DF is to produce H_2 (Bharathiraja et al 2016). The main difference is that DF reduce the methanogens process in favor of H_2 production, where high amounts of VFA is accumulated as a by-product (Chalima et al 2017; Khan et al 2016). VFA can be extracted and re-used, while the remaining residue can be used to produce energy in the form of biohydrogen or biogas (Tampio et al 2018).

2.2.3 Volatile fatty acids

The degradation of food waste produce VFA as an intermediate energy carrying compound. VFA can be produced from any biomass with low lignin content, and consists of multiple short-chain fatty acids such as *acetic acid* ($C_2H_4O_2$), *propionic acid* ($C_3H_6O_2$) and *butyric acid* ($C_4H_8O_2$) (Kim et al 2018; Chalima et al 2020). Research suggest that VFA can be extracted from AD and re-used, for instance as carbon source in feedstock to other organisms (Wainaina et al 2019). However, dark fermentation provides a higher VFA yield per unit food waste (Nordberg, 15 May 2020) and is therefore often used when the aim is to extract VFA. In a study by Chalima et al (2017) VFA was extracted from dark fermentation of food waste and re-used as feedstock to C.Cohnii algae.

2.2.4 Theoretical loss of biogas

Chemical oxygen demand (COD) describes the amount of oxygen (O_2) required for complete chemical decomposition of organic substances. In AD, the COD for food waste is preserved in CO_2 and CH_4. If all biomass is fully biodegradable and converted into biogas, the Buswell equation (see Appendix B) provides a theoretical maximum amount of biogas produced (Buswell & Mueller 1952). Hence, if the composition of VFA removed from AD is known, the Buswell equation and COD approach can be used to calculate the theoretical biogas loss due to VFA removal from the process (Nordberg, 15 May 2020).

2.3 Aquaculture

All farming of aquatic organisms in water environments are refereed to as aquaculture, such as farming salmon and algae (Sprague et al 2015). Today, aquaculture is one of the fastest growing food producing sectors world wide, where about 80 million ton food fish and 89 000 ton algae are produced each year (FAO 2018). Since 2013, the largest single fish commodity by value is salmon and the leading producer is Norway. Farmed salmon accounts for about 35% of the EU's total consumption of aquaculture products (European commission 2019a), while the production of algae (including all species) account for less than 1% (FAO 2018).

Salmon farmed in Norwegian aquacultures are primarily fed dry pellets containing about 25% marine raw material and 75% plant sourced ingredients, where about 10% of the marine ingredients is fish oil (Aas et al 2019; Mowi 2019). Commercial fish oil is mainly produced from wild caught fish, and large environmental impacts arises due to the dependency of marine raw materials (Fréon et al 2017; Merino et al 2014).

Current production methods infer that marine resources decline when the global demand for fish and DHA increase. According to Woods et al (2016), overfishing is the main threat to loss of marine biodiversity as it removes selected species from the ecosystem. Unsustainable aquaculture and fishing still occur (CIWF 2019; FAO 2018), and substantial efforts are urgently required to fulfill current SDGs (Nash 2020). In order to support further growth of aquaculture with less dependency of marine raw materials, alternative ingredients in feed must be used. Since any feedstock containing the required nutrients could be used in salmon feed (Mowi 2019), a solution could be to substitute fish oil with algae oil (Sprague et al 2015).

Algae farming is another sector within aquaculture, which in comparison to salmon is less dependent on marine raw materials. Microalgae is a plant-like organism that grow naturally in marine environments, that is farmed using a so-called starting culture. The starting culture consist of algae cells derived from marine sources (Mendes et al 2009) and additional nutrients depending on algae species. Heterotroph microalgae need to consume carbon to increase in biomass and can therefore be cultivated in closed-pond systems, while autotroph microalgae often requires sunlight for photosynthesis. As suggested by Rösch et al (2018) microalgae can be used within both food and biofuel production, with only minor changes in cultivation and harvesting processes. Although, when algae oil is intended for food industry, mechanical pressing is used to extract oil instead of chemical solvents to prevent toxic residues in food (Silva et al 2019).

2.3.1 DHA produced from fish oil

Fish oil is one of the most important ingredients in conventionally produced fish feed since it provides essential fatty acids (DHA and EPA) and digestible energy (Escudero & Gong 2010). The raw material for fish oil is often wild small fish, such as Peruvian anchovies (Merino et al 2014).

The exploitation of Peruvian anchovy and production of fish oil has been ongoing for over 60 years, and today Peru produce and export 23% of the global demand for fish oil (Fréon et al 2017). According to OECD & FAO (2018) Peru is estimated to remain a leading exporter until 2027 while European countries continue to be main importers of fish oil used for salmon feed. Among all fish species, Peruvian anchovy oil contain one of the highest concentrations of DHA at 10-12% (Ciriminna et al 2019; IFFO 2017b). One contributing factor for the high fish oil yield in Peru is the upwelling of he Humboldt Current outside the Peruvian coast, which enables a high biological productivity (Gutiérrez et al 2016). However, overfishing, climate change and complex weather patterns such as El Niño alter the marine circulation, disrupt biodiversity and can decrease future production of fish oil (SCBD 2017). When simplified, the production process for fish oil rich can be illustrated according to Figure 2 (FAO 1989).

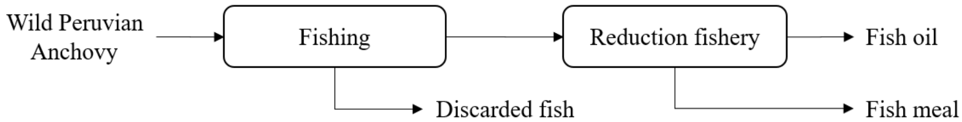


Figure 2: Simplified process for DHA production from fish oil. Illustration and process corresponding to data from IFFO (2017a). Additional energy, transports and processing units are required for this process but excluded in this illustration.

The process begin with *fishing* outside the cost of Peru. Primarily, purse seine boats are used, which operates in the pelagic zone without direct contact with the seafloor (Fréon et al 2017). About 3.9% of the wild catch is not suited for oil production and is therefore discarded to the ocean (Silva et al 2018). The fishing process is often heavily dependent on fossil fuels (Ziegler et al 2016). The catch is transported to the harbor, weighed and via pumps transported to the *reduction fishery* located in the harbour. Reduction fisheries produce fish oil and fish meal from fish via an optimized production process (Fréon et al 2017). In summary, the processing includes hashing and cooking alongside an oil separation process that utilize a screw press to separate liquid from soilds. Furthermore, a centrifuge and a filter are used to separate oil from water and to purify the fish oil. This process requires energy and electricity to produce fish oil rich in DHA and EPA, while the solids can be used to produce fish meal as a by-product (FAO 1986).

2.3.2 DHA produced from microalgae

The algea *Crypthecodinium cohnii* (C.Cohnii) is a heterotroph marine microorganism with an established potential to produce DHA in aquacultures (Mendes et al 2009; Colombo et al 2019). Heterotroph organisms are dependent on organic substrate to increase in biomass (Mendes et al 2009), and one beneficial characteristic of the C.Cohnii is that the only essential fatty acid it produces is DHA (Diao et al 2018). When simplified, the production process for algae oil rich in DHA is illustrated in Figure 3.

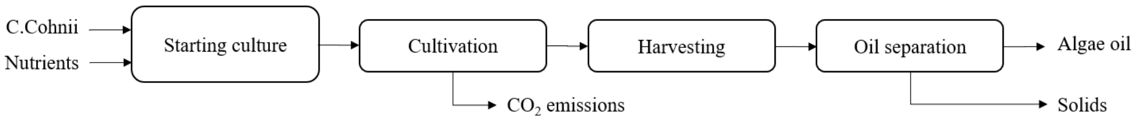


Figure 3: Simplified process for production of DHA rich oil from C.Cohnii microalgae. Additional energy, water and processing units are required for this process but excluded in this illustration.

The *cultivation* of C.Cohnii occurs in absence of light and oxygen, where *nutrients* and C.Cohnii make up a *starting culture* grown in a heterotrophic closed fermenter (Smetana et al 2017). This process includes mixing and temperature control. Nutrients mainly consist of a carbon source, such as molasses or VFA, with an added nitrogen source and salt (Mendes et al 2009). The C.Cohnii are grown in a *pre-cultivation* process, that requires wild algae, fertilizers and water alongside electricity and heat (Smetana et al 2017). Furthermore, less than 1% of C.Cohnii is needed in the starting culture to begin the cultivation process and at the end the algae make up 20% of the culture (Hosseinzadeh-Bandbafha et al 2019; Passell et al 2013). During cultivation about 0.1–4 $kgCO_2$ / kg dry algae is emitted (Silva et al 2019). After the algae is fully grown, the algae culture is pumped to a separate unit to begin *harvesting*. This process includes dewatering to separate algae from water, and in a study by Keller et al (2017) it is stated that most residues and water after harvest can be recovered and re-used. The *oil separation* includes mechanical pressing and a centrifuge process to separate oil from solids (Rösch et al 2018; Taelman et al 2013). The oil content in C.cohnii is about 24% (Mendes et al 2009) with a DHA content ranging from 10-35% depending on feedstock, cultivation and harvest methods (Lemoine & Junne, 5 February 2020; Senanayake & Shahidi 2007). The main product is an algae oil rich in DHA, while the solids are often considered a by-product (Winwood 2013).

2.4 DHA produced from food waste

In recent years, a lot of research has examined different nutrient inputs when using microalgae to produce DHA. One promising area is the use of VFA derived from organic substances, such as food waste. Recent research suggest that traditional two-step AD can be bioenginered to produce more VFA alongside hydrogen, thus upgrading biogas plants into bio-refineries (Wainaina et al 2019). As suggested by Paritosh et al (2017), AD can be used to produce up to 20 gVFA/L food waste, but a higher VFA content will prohibit biogas production. However, a dark fermentation process naturally produce VFA as a by-product and can be used to optimize the extractable VFA content from food waste (Garcia et al 2018; Chalima et al 2017). In a study by Chalima et al (2020) VFA was used as feedstock to produce DHA via C.Cohnii microalgae, thus combining a waste-to-value technique with DHA production. In short, VFA extracted from dark fermentation can in theory be used to support a large scale algae oil production. A simplified process illustration is shown in Figure 4.

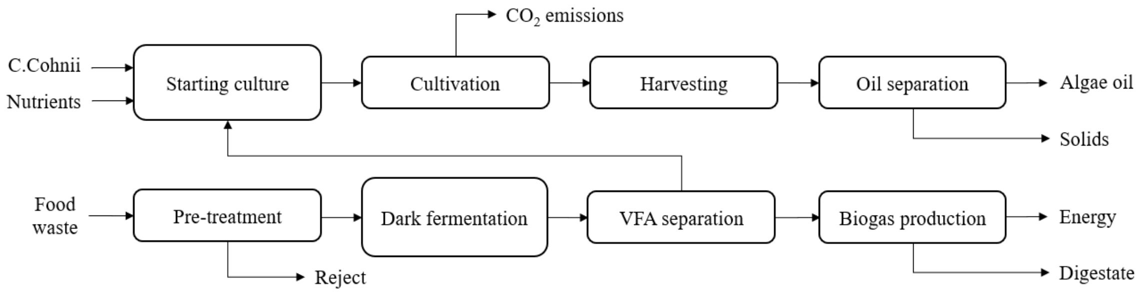


Figure 4: Theoretical DHA production process using VFA from food waste as feedstock to microalgae. Additional energy, water, transports and processing units are required but is excluded in this illustration.

The *pre-treatment* and *dark fermentation* (DF) can be assumed to be similar to the steps in a two-stage AD biogas process, but with a higher VFA content. After DF a *VFA separation* occurs via a series of screw-press liquid hydrolysates and a decanter centrifuge (Jänisch et al 2019; Tampio et al 2018). In a study by Garcia et al (2018) it suggested that up to 25 g VFA per liter household food waste can be produced during optimized dark fermentation, which equals to about 5% VFA per unit food waste. The composition of VFA according to Garcia et al (2018) is about 21-31% acetic acid, 26-49% propionic acid and 17-36% butyric acid. VFA is then pumped to a liquid tanker and transported to the algae production site, while the remaining slurry can be used in biogas production (Tampio et al 2018). The *algae production* is similar to the process illustrated in Figure 3, but with VFA used as a the primary carbon source to enable biomass growth. The main product is an algae oil rich in DHA, while biogas used for energy is a by-product.

2.5 Life cycle assessment

A life cycle assessment (LCA) is a systematic method to develop a holistic picture of environmental impact caused by a process or a product. The impacts are ideally calculated from cradle-to-grave or cradle-to-gate, beginning with extraction of raw material and ending with waste management. Along the way, required materials, energy, transports and emissions are accounted for. In theory, all aspects of inputs and outputs for a given system is considered. In practice, LCA is a model used to describe a simplified version of a complex system. The aim is to identify important and resource-demanding flows and to obtain a quantitative assessment of environmental impact for a given process or product (Klöpffer & Grahl 2014a). In this section, important theory used in an LCA is described.

2.5.1 The LCA approach

There are two approaches used for LCA, attributional (ALCA) and consequential (CLCA). An ALCA comprises inputs and outputs directly associated with the process of interest, while a CLCA is primarily used when a process substitutes another (Bjørn et al 2018; Ekvall et al 2016). The ALCA uses *allocation* for by-products and is preferably used when the aim is to compare two products with the same functional unit (PRé 2016).

According to the ISO 14040/14044 standard (ISO 2016a; ISO 2016b), an LCA consists of the obligatory method elements *classification* and *characterization*, while *normalization* and *weighting* are optional. The LCA framework consists of four iterative phases, illustrated in Figure 5.

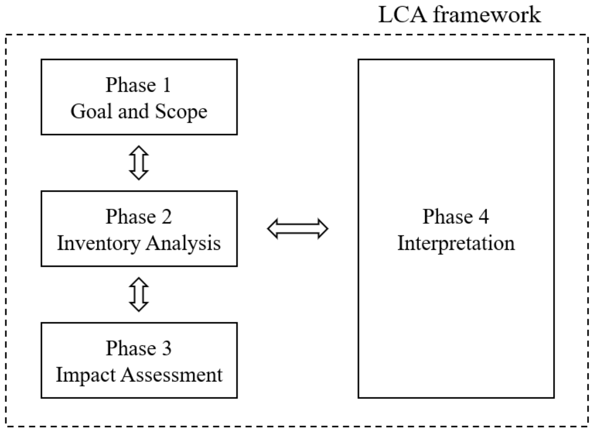


Figure 5: Phases and framework of a LCA according to ISO 14040 (2016a). Figure design inspired by Klöpffer & Grahl (2014a).

In the first phase, the objectives and scope for the study is described. Alongside, *system boundary*, *functional unit* and *impact categories* are selected (Klöpffer & Grahl 2014a). The system boundary determines which activities and processes are included or excluded in the LCA process. The functional unit (FU) is a reference unit to which all flows are related, for instance */ton product*. Impact categories represent environmental issues of concern in regard to the selected system, such as climate change and fossil energy use.

In the second phase, an inventory analysis (LCI) is performed where the parameters for inputs and outputs are quantified through a data collection process. Examples of inputs and outputs are the amount materials required, energy used and emissions linked to a certain system. In most processes, both a main product and by-products are produced. *Allocation* and *system expansion* are the main methods for estimating how resources and emissions are distributed between these products (Klöpffer & Grahl 2014b).

In the third phase, the impact assessment (LCIA) is conducted, where inventory data (LCI) is translated into environmental impact. This is done by using an LCIA method for *classification* and *characterization*. During classification, all emissions are sorted into groups depending on their environmental effects at either *midpoint* or *endpoint* level (Klöpffer & Grahl 2014c; Meijer 2015). Characterization quantify the classified emissions impact by multiplying with a characterisation factor, specific for the selected LCIA method. In this stage a numerical result for environmental impact is obtained, expressed in the FU. Lastly, a *sensitivity analysis* is preformed to assess the importance of assumptions and the reliability of the results (Klöpffer & Grahl 2014c).

Since an LCA is an iterative process, the fourth phase infer that assumptions and results are continuously analyzed and adjusted with regard to the objective and scope. When the LCA is finished, the results can be used by decision makers and industry to promote sustainable development. The results can also be used to compare two products or scenarios, provided that chosen system boundaries and assumptions allow a fair comparison, and that they are quantified by the same functional unit (Ruiza & Ghorabi 2010).

2.5.2 Impact categories

Research shows that current aquaculture contribute to global warming, dependency on fossil fuels and loss of biodiversity (Silva et al 2018; Ziegler et al 2016; Muir 2015). Meanwhile, future food and DHA production are at risk when natural ecosystems are damaged (Colombo et al 2019). This demonstrates the importance of assessing *global warming*, *abiotic depletion potential, fossil fuels* as well as *Ecosystem damage*. Two LCIA methods that assess these impact categories are CML-IA and ReCiPe Endpoint (Pré 2020). Both methods use data from IPCC with a timeline of 100 years to assess global warming and climate change (Rosenbaum 2018).

2.5.3 Impact assessment methods

When LCI data is collected, it is translated into environmental impact via LCIA methods. Generally, impact assessment can be preformed at either midpoint or endpoint level during the cause-effect chain (Meijer 2015). Midpoint methods are used to assess a specific issue, such as global warming, by converting LCI into a common unit for each impact category. Endpoint methods combine multiple impacts on an area of protection, such as ecosystem damage, by aggregating impact categories into a common unit. According to Rosenbaum et al (2018), the result from midpoint methods is more precise but less environmentally relevant in comparison to endpoint methods. Endpoint methods, on the other hand, include more choices and aggregating of results during modelling, which causes an increased uncertainty in the numerical result. Therefore, a thorough sensitivity analysis should be preformed before conclusions are drawn (Coustillas, 14 April 2020).

CML-IA is a LCIA method developed by Center of Environmental Science of Leiden University (Pré 2020). The impact category *global warming* is expressed in carbon dioxide equivalents ($kgCO_2e$), and serve as an indicator of global warming potential with a 100 year timeline (GWP_{100}). *Abiotic depletion potential* ($ADP_{fossil fuels}$) is expressed in megajoule (*MJ*) and serve as an indicator for extraction of non-renewable resources (Rosenbaum 2018). As illustrated in Figure 6, GWP_{100} includes contribution from three GHG emissions while $ADP_{fossil fuels}$ include direct and indirect contributions from extraction of oil, coal and natural gas.

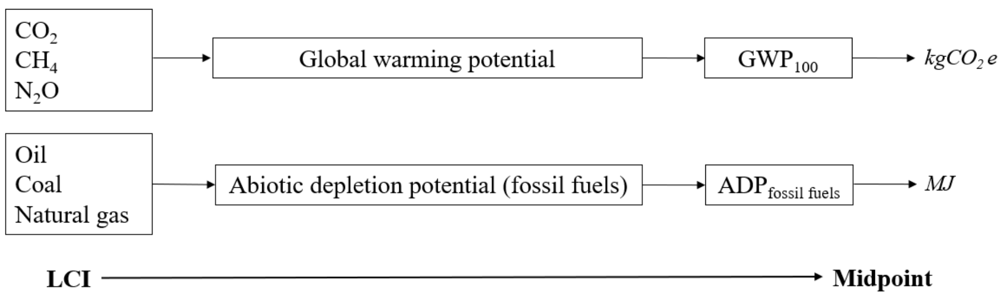


Figure 6: Illustration of the CML-IA midpoint impact assessment.

ReCiPe 2016 is the latest version of the method developed by Radboud University Nijmegen and PRé Sustanabliuty. ReCiPe Endpoint (H) uses the *Hierarchist* setting to assess damage impact by using multiple midpoint categories to provide a single score for damage impact, such as *Ecosystem damage* illustrated in Figure 7. The unit describes a potential in disappeared fraction of species per year (species.yr) (Goedkoop 2016).

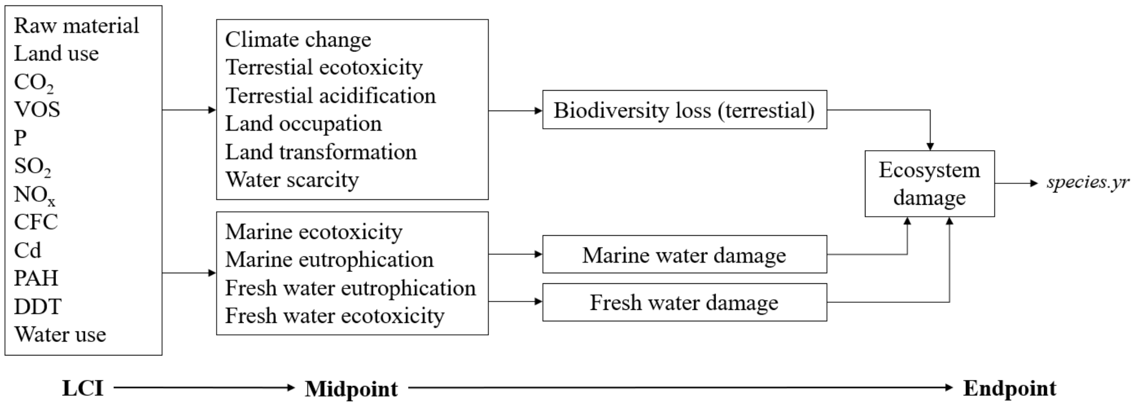


Figure 7: Illustration of the ReCiPe damage assessment at endpoint for Ecosystem damage. Figure design inspired by Goedkoop (2016).

Species in ReCiPe refer to plants and microorganisms that support terrestrial and aquatic food chains. Their extinction will affect higher organisms, but the direct disappearance of higher organisms is not modelled. Additionally, ReCiPe treat all species equal and does not account for endangered species or direct biodiversity effects (Piekema, 7 April 2020).

2.5.4 System expansion and allocation

There are two ways to allocate the environmental burden between by-products from the same process in an LCA, by using *system expansion* or *allocation*. The principle of system expansion is to include the use of by-products into the system boundaries, by assuming that by-products replace a product or service on the global market. This method is preferably used when conventional data can easily be identified, such as energy (Bjørn et al 2018). For instance, if biogas is used to produce electricity it can be assumed to replace electricity from the national energy grid. Moreover, system expansion is considered more scientific in comparison to allocation (Klöpffer & Grahl 2014b). If system expansion can not be preformed, the ISO 14044 suggest that allocation is applied (Bjørn et al 2018).

The principle of allocation is to divide the environmental impact between products and by-products, depending on their mass or economic value. Although, if the by-product is negligible with regard to mass or economic value in comparison to the main product, the entire impact is addressed to the main product (Bjørn et al 2018). For instance, the digestate produced alongside biogas (see Figure 1) is often regarded as waste in LCA due to the low market value. Some databases provide pre-allocated datasets, while other processes require additional allocation or system expansion.

2.5.5 Calculation methods

Ecoinvent is the most widely used database for supply chain and background data for LCA (Ecoinvent 2020a). The latest version, Ecoinvent 3.5, includes Peruvian site locations and extended datasets for fishing (Avadí et al 2019). The datasets can be used with different allocation settings, for instance *Allocation, cut-off by classification*. This setting provides data related to the main product (Ecoinvent 2020b) and ensure that ALCA modelling principles are applied (PRé 2016). Alongside, additional transport data between production sites is often required. The calculation tool NTMCalc, developed by the Swedish Network for Transport Measures, can estimate transport distances and emissions (NTM 2019).

SimaPro is an established LCA modelling software developed by PRé Consultants (PRé 2020) that provides a transparent and systematic way to assess complex life cycles. The software follows the ISO 14040/14044 standard and includes several inventory databases and impact methods (PRé 2016). SimaPro 9 is the latest version of the software, which includes Ecoinvent 3.5, CML-IA and ReCiPe 2016 (PRé 2020).

2.5.6 Biodiversity in LCA

All fishing activities cause direct impact on biodiversity through direct biomass removal and diminished ecosystem functions (Langlois et al 2014). This impact can consequently not be fully quantified through resource use or emissions. However, current accessible LCA methods only include indirect aspects of biodiversity, such as emissions and land use. Moreover, only *habitat loss*, *climate change* and *pollution* of the five identified drivers for biodiversity loss are covered in available methods (Winter et al 2017). In summary, all direct effects alongside *overexploitation* and *invasive species*, lack standardized application within LCA (Crenna et al 2018; Woods et al 2016).

Research is currently developing methods to obtain a more holistic impact assessment for biodiversity (Avadí et al 2019). Some examples of relevant research are *Marine biotic resource depletion* (Hélias et al 2018), *Overfishing* (Emanuelsson et al 2014), *Invasive species* (Hanafiah et al 2013), *Product Biodiversity Footprint* (Asselin et el 2020) and *Naturalness* (Farmery et al 2017). Although, no consensus has yet been reached to use a specific method (FAO 2015) which means that impact on biodiversity cannot be fully assessed. One established LCIA method that addresses some impacts on biodiversity is the ReCiPe Endpoint method for Ecosystem damage.

3 Method

This thesis assess the environmental impact of DHA produced by the heterotroph microalgae C.Cohnii using VFA extracted from food waste. In order to evaluate the process in comparison to existing production methods, a reference scenario for DHA produced from Peruvian anchovy oil was assessed. This thesis combine production methods for biogas, VFA production via dark fermentation, algae oil and fish oil to model a large scale production of DHA. An attributional LCA according to ISO standards was conducted for each scenario and described in this section. Pre-allocated data from Ecoinvent 3.5 was used for the background system, wheras system expansion was used in the foreground system for the main by-product biogas. The studied systems were modelled as two parallel scenarios: a conceptual *Algae scenario* where DHA was produced from C.Cohnii microalgae using VFA from food waste, and a conventional *Fish scenario* where DHA was derived from Peruvian anchovy. A physical functional unit (tonDHA) was chosen to enable comparison between DHA produced from algae oil and fish oil (Ruiza & Ghorabi 2010). Supporting calculations for LCI data are found in Appendix A.

3.1 Methodical choices

The system represent a cradle-to-gate LCA where the system boundaries start with extraction of raw materials and end when the oil arrive at the salmon feed manufacture gate. An overview of the assessed production process for oil rich in DHA is illustrated in Figure 8.

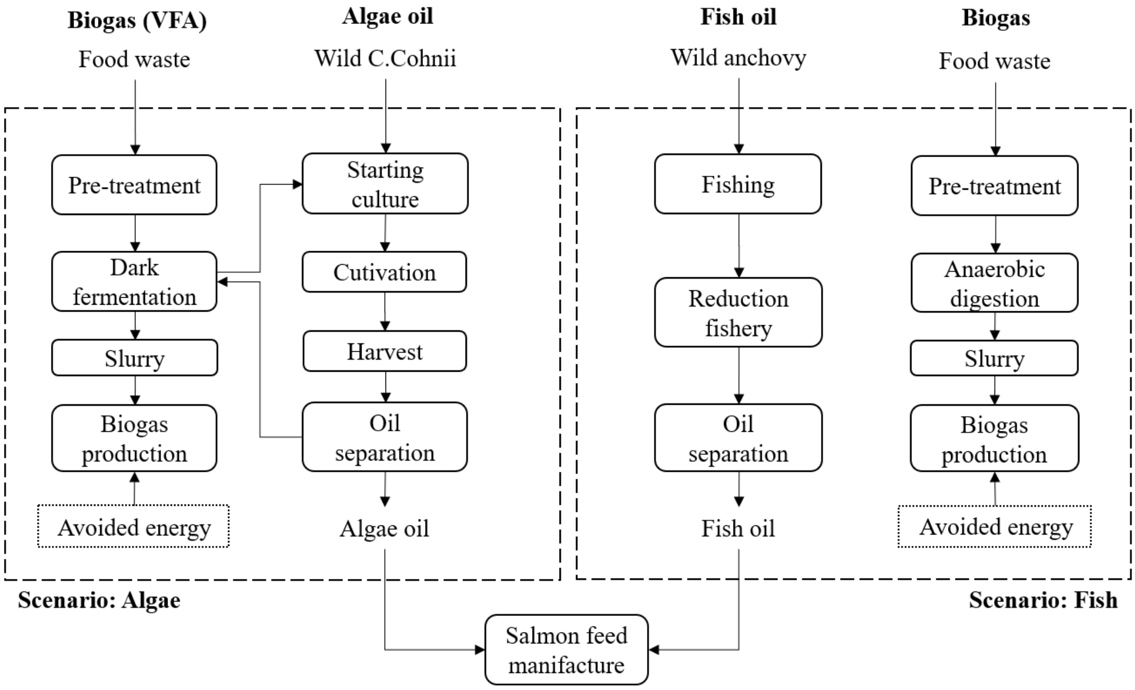


Figure 8: A process overview of assessed DHA production, illustrating the comparison between the Algae scenario and Fish scenario. The system boundary is illustrated as a dashed line and the dotted lines surround by-products included via system expansion.

Included in the system was processing of material, use of inputs and energy, as well as construction of buildings and use of machinery for processing. Construction and maintenance of additional infrastructure are out of the scope in this study. Production and end of life for inputs required were considered. Transport was included for inputs and outputs, while intermediate transport at production site was excluded.

Within the AVARE project, a small-scale waste-to-value technique for DHA produced from food was developed in Berlin. Therefore, the site location for algae oil and biogas production was assumed to be Berlin, Germany. Meanwhile, Peru is the leading exporter of fish oil used in aquaculture (OECD & FAO 2018). The fish oil production was therefore assumed to be located in Lima, Peru. Primarily, site specific data was used which provides a site dependent result. German energy mix, primarily sourced from oil, coal and natural gas (IEA 2018), was used for algae oil and biogas production. However, according to Klaus et al (2010) until 2050 the aim 100% renewable energy derived from wind power, photovoltaicis and biomass.

Food waste delivered at the biogas plant was considered a free resource and did not contribute to environmental impact, since the production belong to the preceding food system. The digestate produced alongside biogas was assumed to have a negligible market value in comparison to biogas (Klackenberg 2019) but could be re-used as fertiliser. Therefore, the transport from biogas production was included but not end of life for digestate. The inventory analysis was performed using Excel and SimaPro 9 (PhD licence) was used to model impact assessment. NTMCalc Basic 4.0 was used to estimate transport distances for inputs and outputs, and a 3.5 ton freight lorry with Euro VI was assumed for most transport. By-products were assessed via system expansion and the electricity generated from biogas production was assumed to replace German energy mix.

3.2 DHA produced from algae oil

This scenario comprises DHA production via C.Cohnii using VFA from food waste as feedstock, illustrated in Figure 9. The main product obtained was algae oil rich in DHA while biogas was considered a by-product.

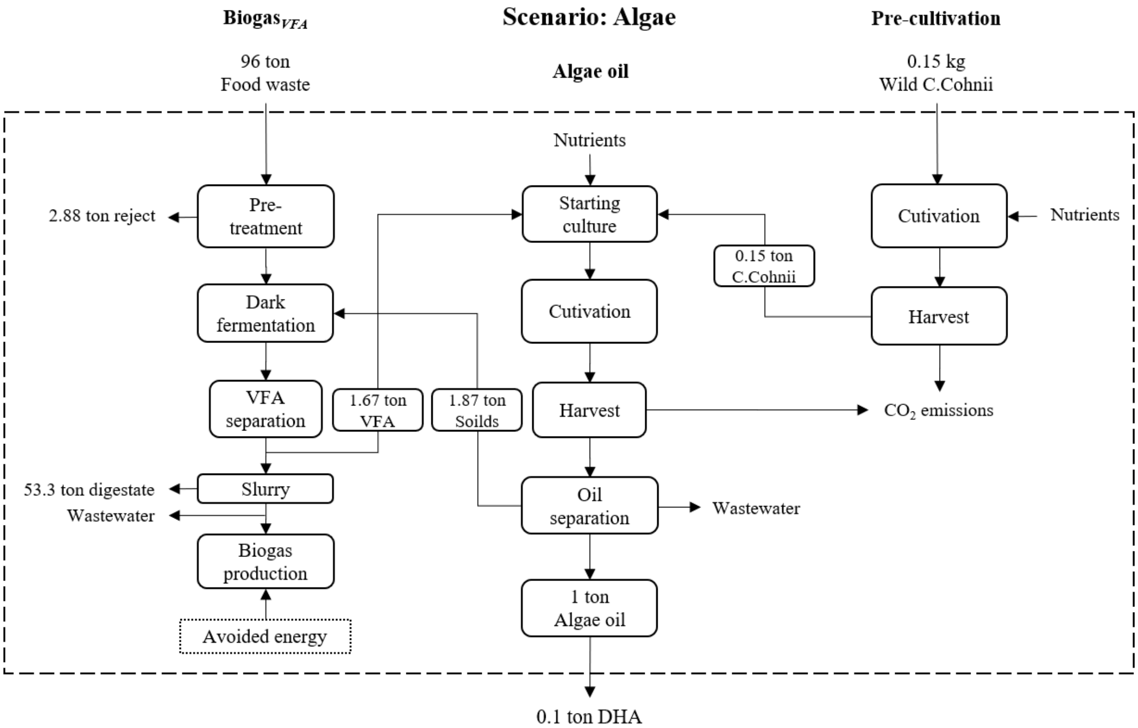


Figure 9: The Algae scenario includes production of algae oil and biogas with VFA extraction. Additional energy and water is needed but excluded in this illustration.

3.2.1 Inventory analysis: Algae scenario

Inputs and outputs for pre-cultivation of C.Cohnii was assumed to correspond with inputs suggested by Hosseinzadeh-Bandbafha et al (2019) and Smetana et al (2017). According to Hosseinzadeh-Bandbafha et al (2019), 1% wild algea was required to produce 100% grown, harvestable algea, which implies that 150 kg required in the present study would need 0.15 kg wild C.Cohnii. The production of algae oil was assumed to be similar to algae production, but using VFA extracted from dark fermentation as carbon source. VFA was considered a limiting factor and the amount required for algae growth was suggested by Lemoine & Junne (5 February 2020), which was then scaled up to represent an large scale production. Assuming that 1.8% VFA could be extracted in dark fermentation, 93 ton pre-treated food waste was needed to produce 1.67 ton VFA. The amount of CO_2 emitted from heterotrophic algae cultivation was set to 0.1 kg CO_2/ kg C.Cohnii since carbon primarily was used as energy (Silva et al 2019). The algae solid residue from oil separation was assumed to be re-used in dark fermentation to generate biogas. Processing needed for pre-cultivation and oil separation was modelled based on the amount of substance treated, while the processing for cultivation and harvest was divided by two to represent a smaller unit. In the processing dataset, machinery and buildings was included. Table 1 provide LCI inputs and state which Ecoinvent dataset was used.

Table 1: Inventory data for algae oil, expressed per ton algae oil.

Pre-cultivation		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Wild C.Cohnii	1,50E-01	kg	Appendix A.1.2	1
	N fertiliser	3,90E+00	kg	Appendix A.1.2	2
	P fertiliser	6,75E-01	kg	Appendix A.1.2	3
	Transport	3,00E+01	km	n/a	4
	Fresh water	2,51E+01	m^3	Appendix A.1.2	5
	Electricity	1,28E+03	kWh	Appendix A.1.2	6
	Processing	1,50E-02	m^3	n/a	7
	Flue gas	-2,72E+00	ton	Appendix A.1.2	8
Output	C.Cohnii	1,50E-02	ton	Appendix A.1.2	n/a
	CO_2 to air	1,50E-02	kg	Silva et al (2019)	9
	Wastewater	2,50E+01	m^3	n/a	10
Starting culture		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	VFA	1,67E+00	ton	Appendix A.1.1	n/a
	C.Cohnii	1,50E-02	ton	Appendix A.1.1	n/a
	Yeast	3,00E-02	ton	Appendix A.1.1	11
	Reef salt	3,75E-01	ton	Appendix A.1.1	12
	Molasses	1,35E-01	ton	Appendix A.1.1	13
	Fresh water	1,23E+01	m^3	Appendix A.1.1	5
	Transport	1,00E+02	km	n/a	4
Cultivation & Harvest		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Electricity	1,28E+03	kWh	Hosseinzadeh et al (2019)[2]	6
	Heat	1,12E+07	BTU	Hosseinzadeh et al (2019)[2]	14
	Processing	7,28E+00	m^3	n/a	7
Output	CO_2 to air	1,50E-03	ton	Silva et al (2019)	9
Oil separation		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Electricity	2,21E+03	kWh	Hosseinzadeh et al (2019)[2]	6
	Heat	8,22E+06	BTU	Hosseinzadeh et al (2019)[2]	14
	Processing	1,46E+01	m^3	n/a	7
Output	Algae oil	1,00E+00	ton	Hosseinzadeh et al (2019)[2]	n/a
	Algae solids	1,87E+00	ton	Hosseinzadeh et al (2019)[2]	n/a
	Wastewater	1,17E+01	m^3	n/a	10
Transport to feed manifacture		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Berlin (Germany) to Sjøhagen 15 (Norway)		1,86E+03	km	NTMCalc (2020)	4

[1] Ecoinvent datasets used in SimaPro, see Appendix D

[2] Hosseinzadeh-Bandbafha et al (2019)

The LCI for biogas via dark fermentation in Table 2 was assumed to be similar to AD biogas production, with additional VFA separation. $Biogas_{VFA}$ represent biogas production using dark fermentation with VFA extraction. 96 ton collected food waste was required to support algae cultivation, when assuming a 3% loss during pre-treatment. 50% of the reject was assumed plastic and 50% aluminum, where plastic was re-used in municipal waste incineration and aluminum re-cycled. Solids from algae production was re-used in biogas production, and assumed to have equivalent potential to generate biogas as food waste. The extracted VFA was assumed to be fully biodegradable. Maximum biogas loss, due to removal of carbon in the form of VFA, was calculated using COD and Buswell equation. The amount of slurry from DF was not altered during modelling, since the loss of biogas was subtracted later. From the remaining slurry, 60% was used to produce biogas and 40% was digestate. The biogas production was assumed to have 90% efficiency rate with 60% CH_4 content (World biogas association 2019). The processing represent buildings and additional machinery, and was calculated depending on the dataset unit. LCI for pre-treatment processing was calculated by dividing the processing needed with its lifetime capacity. DF processing was obtained by adding corresponding amount of inputs, assuming that 1 ton slurry occupies 1 m^3, while VFA processing correspond to the amount of VFA extracted. The amount of energy needed to produce energy from biogas, the biogas processing step, was assumed equal to the amount of produced energy.

Table 2: Inventory data for Biogas_{VFA}, expressed per ton algae oil.

Pre-treatment		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset**[1]
Input	Collected FW	9,60E+01	ton	n/a	n/a
	Transport	3,00E+01	km	n/a	15
	Processing	1,92E-04	unit	n/a	16
Output	Metall reject	1,44E+00	ton	Appendix A.1.3	17
	Plastic reject	6,68E+04	MJ	Appendix A.1.3	18
	Transport	3,00E+01	km	n/a	4
Dark fermentation		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset**[1]
Input	Pre-treated FW	9,30E+01	ton	n/a	n/a
	Algae solids	1,87E+00	ton	Hosseinzadeh et al (2019)[2]	n/a
	Transport	1,00E+01	km	n/a	4
	Water	5,32E+01	m^3	Opatokun et al (2017)	19
	Electricity	7,59E+02	kWh	Opatokun et al (2017)	6
	Procesing	1,48E+02	m^3	n/a	20
Output	Slurry from DF	1,48E+02	ton	n/a	n/a
VFA extraction		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset**[1]
Input	Slurry from DF	1,48E+02	ton	n/a	n/a
	Electricity	2,10E+02	kWh	Passell et al (2013)	6
	Processing	1,67E+00	ton	n/a	21
Output	VFA	1,67E+00	ton	Appendix A.1.1	n/a
	Biogas slurry	1,48E+02	ton	n/a	n/a
Biogas production		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset**[1]
Input	Biogas slurry	1,48E+02	ton	n/a	n/a
	Processing	5,49E+04	kWh	Appendix C	22
Output	Biogas	1,16E+04	m^3	Appendix C	n/a
	Biogas loss (VFA)	-1,44E+03	m^3	Appendix B	n/a
	Digestate	5,33E+01	ton	n/a	n/a
	Transport	2,00E+01	km	n/a	4
	Wastewater	0,00	m^3	n/a	10
System expansion		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset**[1]
	Avoided energy	5,49E+04	kWh	Appendix C	6

[1] Ecoinvent datasets used in SimaPro, see Appendix D

[2] Hosseinzadeh-Bandbafha et al (2019)

3.2.2 Impact assessment: Algae scenario

The impact assessment was preformed using CML-IA at midpoint and ReCiPe Endpoint (H) at endpoint. Environmental impact per ton oil was obtained by adding LCIA data from Algae oil and Biogas$_{VFA}$. The impact expressed per tonDHA produced from algae oil (DHA$_{algae}$) was obtained via division by 0.1 (DHA content of 10%).

3.3 DHA produced from fish oil

This scenario comprises production of fish oil in reduction fisheries using wild Peruvian anchovy, while food waste was used to produce biogas in Germany, see Figure 10. In a recent LCA study by Silva et al (2018) it is stated that 22.2 ton wild Peruvian anchovies are required to produce 1 ton fish oil. The main product obtained was fish oil rich in DHA while biogas was considered a by-product.

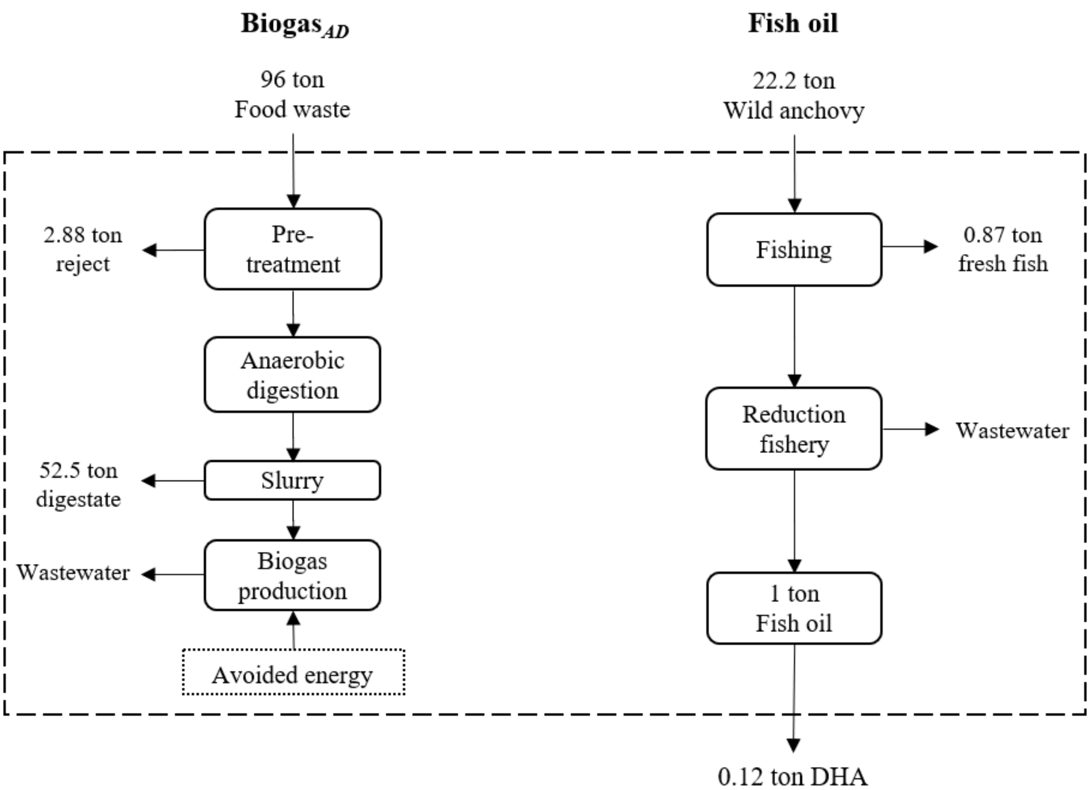


Figure 10: The Fish scenario includes production of fish oil and biogas. Additional energy and water is needed but excluded in this illustration.

3.3.1 Inventory analysis: Fish scenario

The production of fish oil was conducted according to Figure 2 and numeric values are used in accordance with Silva et al (2018). Pre-allocated data from Ecoinvent for fishing and reduction fishery processing was used and no by-products were produced. In the processing dataset, required machinery and buildings were included. Table 3 contain LCI data used for fish oil production.

Table 3: Inventory data for fish oil, expressed per ton fish oil.

Fishing		Amount	Unit	Reference	Ecoinvent dataset[1]
Input	Wild anchovy	2,22E+01	ton	Silva et al (2018)	23
Output	Landed anchovy	2,13E+01	ton	Silva et al (2018)	n/a
	Discarded fish	8,66E-01	ton	Silva et al (2018)	n/a
Reduchtion fishery		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Landed anchovy	2,13E+01	ton	Silva et al (2018)	n/a
	Processing	2,13E+01	ton	n/a	24
Output	Fish oil	1,00E+00	ton	Silva et al (2018)	n/a
Transport to feed manifacture		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Lima (Peru) to Caracas (Venezuela)		4,28E+03	km	NTMCalc (2020)	25
Caracas (Venezuela) to Gravane 4 (Norway)		8,14E+03	km	NTMCalc (2020)	26
Gravane 4 (Norway) to Sjøhagen 15 (Norway)		2,31E+02	km	NTMCalc (2020)	4

[1] Ecoinvent datasets used in SimaPro, see Appendix D

The production of Biogas$_{AD}$ assumes the same inputs and processing calculations as described in Biogas$_{VFA}$, but without the VFA extraction and addition of algae solids to AD. Table 4 shows LCI used for Biogas$_{AD}$ production.

Table 4: Inventory data for Biogas$_{AD}$, expressed per ton fish oil.

Pre-treatment		Amount	Unit	Reference	Ecoinvent dataset[1]
Input	Collected FW	9,60E+01	ton	n/a	n/a
	Transport	3,00E+01	km	n/a	15
	Processing	1,92E-04	unit	n/a	16
Output	Metall reject	1,44E+00	ton	Appendix A.1.3	17
	Plastic reject	6,68E+04	MJ	Appendix A.1.3	18
	Transport	3,00E+01	km	n/a	4
Anaerobic digestion		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Pre-treated FW	9,30E+01	ton	n/a	n/a
	Water	5,29E+01	m^3	Opatokun et al (2017)	19
	Electricity	7,44E+02	kWh	Opatokun et al (2017)	6
	Procesing	1,46E+02	unit	n/a	20
Output	Biogas slurry	1,46E+02	ton	n/a	n/a
Biogas production		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
Input	Biogas slurry	1,46E+02	ton	n/a	n/a
	Processing	6,15E+04	unit	Appendix C	22
Output	Biogas	1,14E+04	m^3	Appendix C	n/a
	Digestate	5,25E+01	ton	n/a	n/a
	Transport	3,00E+01	km	n/a	4
	Wastewater	3,87E-01	m^3	n/a	10
System expansion		**Amount**	**Unit**	**Reference**	**Ecoinvent dataset[1]**
	Avoided energy	6,15E+04	kWh	Appendix C	6

[1] Ecoinvent datasets used in SimaPro, see Appendix D

3.3.2 Impact assessment: Fish scenario

The impact assessment was conducted using CML-IA at midpoint and ReCiPe Endpoint (H) at endpoint. Environmental impact per ton oil was obtained by adding LCIA data from Fish oil and Biogas$_{AD}$. The impact expressed per tonDHA produced from fish oil (DHA$_{fish}$) was obtained via division by 0.12 (DHA content of 12%).

3.4 Sensitivity analysis

Three sensitivity analyses were conducted to identify uncertainties in the result, and were selected with regard to required assumptions and development potential.

3.4.1 Future energy mix

Sustainable development involves shifting from fossil energy to renewable and Germany, among many countries, has the ambition to be 100% renewable until 2050. Alongside, one important aspect of biogas used to produce energy is to reduce dependency on fossil energy (World Biogas Association 2018). If energy is instead coming from renewable sources, such as wind, solar and biomass, some benefits attributed to biogas might shift (IEA 2020; Klaus et al 2010). Therefore, a future energy mix was relevant to study. This was accomplished by changing avoided energy in system expansion, from 100% German energy mix to a mix of wind power, photovoltaic and biomass according to Table 5.

Table 5: Inventory data for avoided energy, assuming a future energy mix.

Biogas$_{VFA}$	**Amount**	**%**	**Unit**	**Ecoinvent dataset**[1]
Biomass	5,49E+03	10	kWh	2,2E+01
Wind power	2,47E+04	45	kWh	2,7E+01
Photovoltaics	2,47E+04	45	kWh	2,8E+01
Biogas$_{AD}$	**Amount**	**%**	**Unit**	**Ecoinvent dataset**[1]
Biomass	6,15E+03	10	kWh	2,2E+01
Wind power	2,77E+04	45	kWh	2,7E+01
Photovoltaics	2,77E+04	45	kWh	2,8E+01

[1] Ecoinvent datasets used in SimaPro, see Appendix D

3.4.2 Optimized VFA content

Research suggests that dark fermentation can be optimized to produce more VFA per unit organic waste (Strazzera et al 2018). In a study by Garcia et al (2018) 25 g VFA/ L food waste was produced, which can be assumed to represent 5% VFA content. If less food waste is required to produce the same amount of VFA, the amount of algae oil produced per unit food waste would increase which could alter the assessed environmental impact. According to United Nations (2019) an optimized production process can promote sustainable resource use and energy efficiency, which support SDGs. Therefore, an optimized dark fermentation process was relevant to investigate. This was accomplished by re-calculating LCI for Biogas$_{VFA}$ and Biogas$_{AD}$, assuming that instead of 5% VFA could be extracted per unit food waste. To generate 1.67 ton VFA 33 ton pre-treated food waste was required.

3.4.3 Increased transport

Transport related emissions cause about a quarter of the GHG emissions in Europe, which contribute to global warming and increased pollution (European Commission 2016). In this thesis, the shortest transport distance according to NTMCalc was assumed for both scenarios, making a sensitivity analysis for increased transports relevant to study. This was accomplished by increasing all LCI transport distances with 10%.

4 Results

This section provides results for assessed environmental impact and each sensitivity analysis. See Appendix E for a visual representation of the SimaPro model set-up.

4.1 Environmental impact: Algae scenario and Fish scenario

The result obtained for the production scenario Algae and Fish, expressed per ton oil, is presented in this section. The result show that when environmental impact for each production process in respective scenario was added, the Algae scenario infer -1.9E-01 TJ fossil energy use, -1.9E+01 tonCO_2e and 9.7E-05 species.yr per ton oil. The Fish scenario requires 1.6E-01 TJ, 1.5E+01 tonCO_2e and 2.0E-04 species.yr. Assessed environmental impact for algae oil and fish oil with respect to $ADP_{fossil fuels}$, GWP_{100} and Ecosystem damage are shown in Table 6.

Table 6: Environmental impact per ton oil for Algae scenario and Fish scenario.

Assessed impact	Unit	Scenario: Algae	Scenario: Fish
$ADP_{fossil\ fuels}$	TJ / ton oil	-1,9E-01	1,6E-01
GWP_{100}	tonCO_2e / ton oil	-1,9E+01	1,5E+01
Ecosystem damage	species.yr / ton oil	9,7E-05	2,0E-04

For ecosystem damage, the Algae scenario infer about 66% lower impact in comparison to the Fish scenario for every ton oil produced. Supporting results are found in Appendix F.1 and Appendix F.2.

4.1.1 Global warming and fossil fuels

With respect to *Abiotic depletion potential*, the result shows that fish oil infer ten times higher environmental impact in comparison to algae oil. In detail, cultivation and harvest constitutes 58% of the total impact from algae oil production, while transports make up about 30%. For fish oil, $ADP_{fossil fuels}$ from reduction fishery processing contribute to 88% of total impact, while fishing about 4% and transport less than 8%. The highest impact from biogas production, with and without VFA extraction, was the pre-treatment and avoided energy. The pre-treatment make up just over 12%, while avoided energy for $Biogas_{VFA}$ was 76% and for $Biogas_{AD}$ over 80%. The additional VFA separation process contribute to about 7% for $Biogas_{VFA}$. In summary, the highest contributing factor with respect to $ADP_{fossil fuels}$ was reduction fishery processing and avoided energy from $Biogas_{AD}$, see Figure 11.

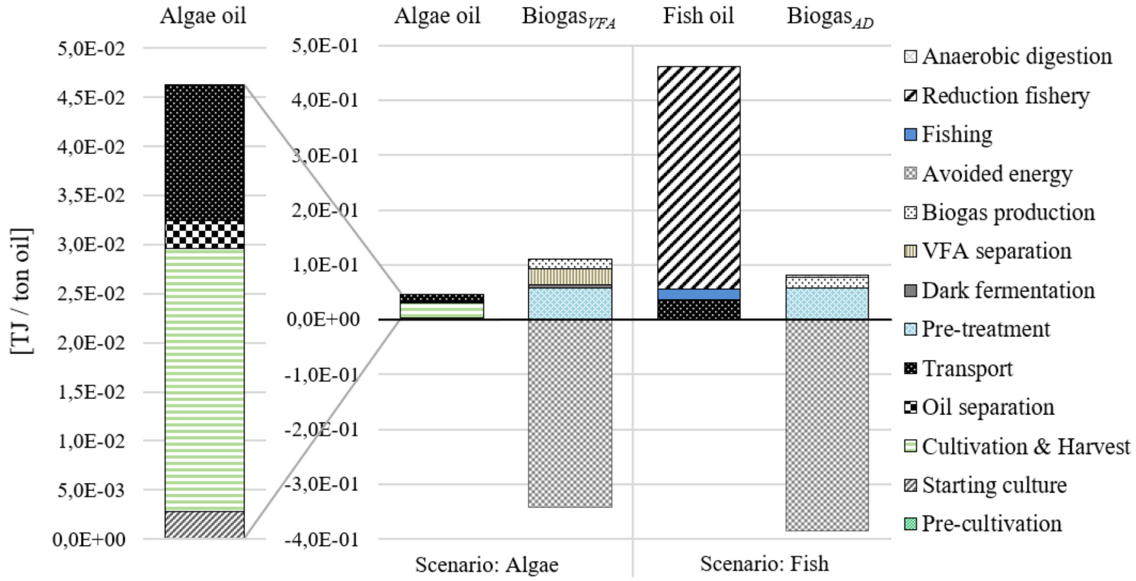


Figure 11: Environmental impact with respect to ADP$_{fossil fuels}$. The results show impact for the Algae and Fish scenario, expressed in TJ per ton oil.

With respect to *global warming potential*, fish oil infer about ten times higher environmental impact in comparison to the algae oil, see Figure 12. Reduction fishery processing make up over 91% of total environmental impact from fish oil, fishing 3% and transport about 8%. For algae oil production, cultivation and harvest constitutes 66% of the total impact while transports was about 22%. Oil separation and starting culture was about 6-7% each. The highest impact from both biogas production processes was pre-treatment and avoided energy. The pre-treatment represent about 9% for both, while avoided energy contribute to 75% for Biogas$_{VFA}$ and 82% for Biogas$_{AD}$. For Biogas$_{VFA}$ the VFA separation was the third largest contributor to environmental impact.

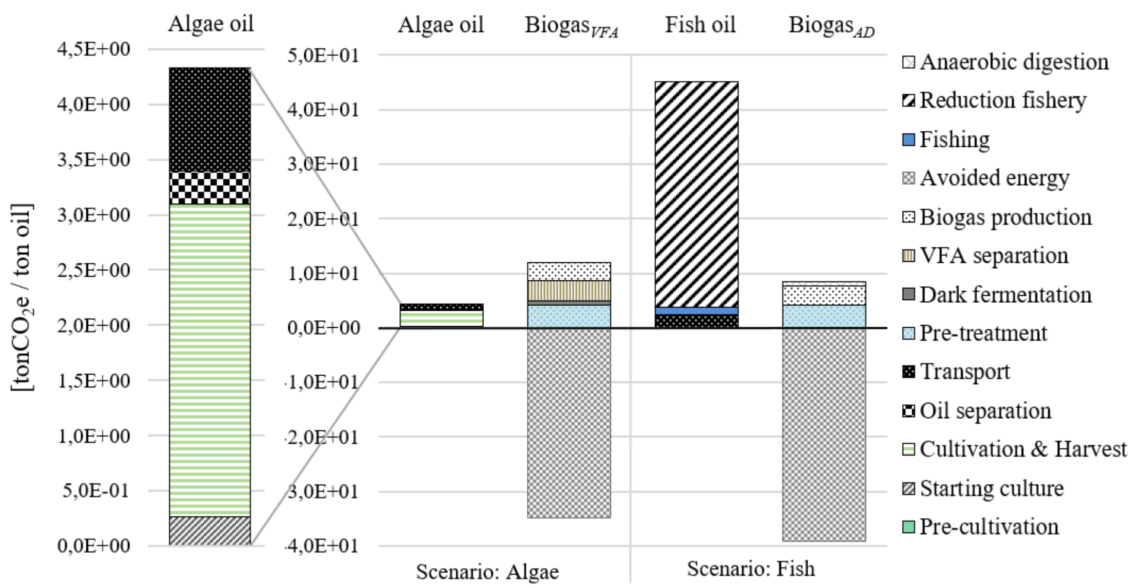


Figure 12: Environmental impact with respect to GWP_{100}. The results show impact for the Algae and Fish scenario, expressed in $tonCO_2e$ per ton oil.

4.1.2 Effects on biodiversity

The results with respect to Ecosystem damage show that environmental impact from fish oil was more than twelve times higher in comparison to algae oil. For algae oil, cultivation and harvest infer 67% of total impact, where global warming, acidification and land use was the main contributing factors. The impact from fish oil was 91% from reduction fishery processing, where global warming, acidification and ozone formation contribute the most to indirect Ecosystem damage. Biogas production and avoided energy leads to decreased global warming and eutrophication, but increased acidification. For Biogas$_{VFA}$, the VFA separation process contribute to about 32% of total Ecosystem damage and cause increased land use. The results are visualized in Figure 13 and 14.

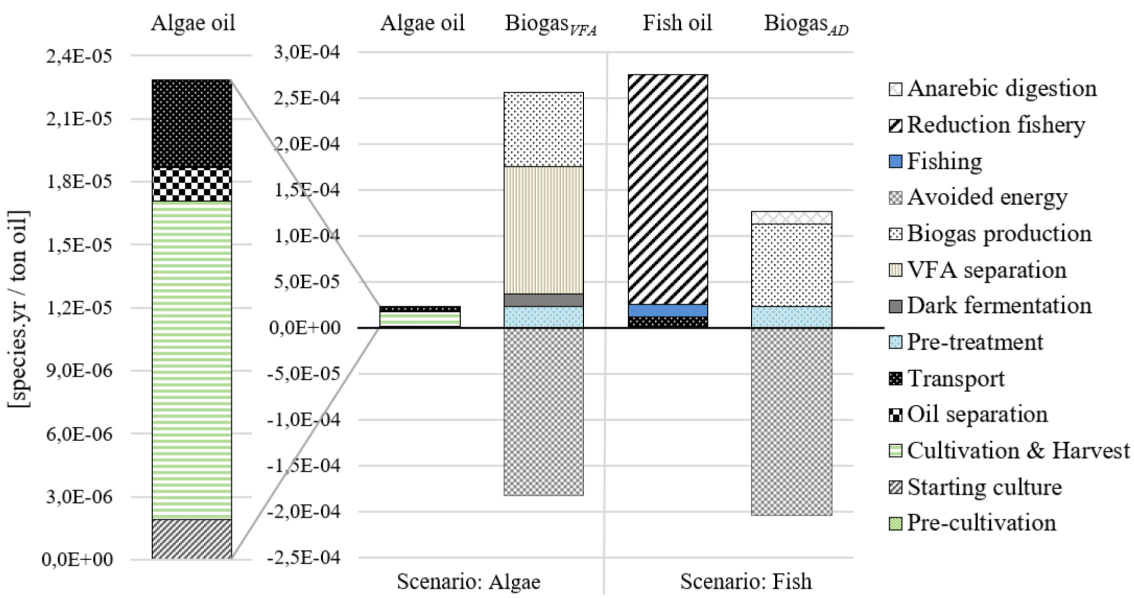


Figure 13: Environmental impact with respect to Ecosystem damage. The results show impact for the Algae and Fish scenario, expressed in species.yr per ton oil.

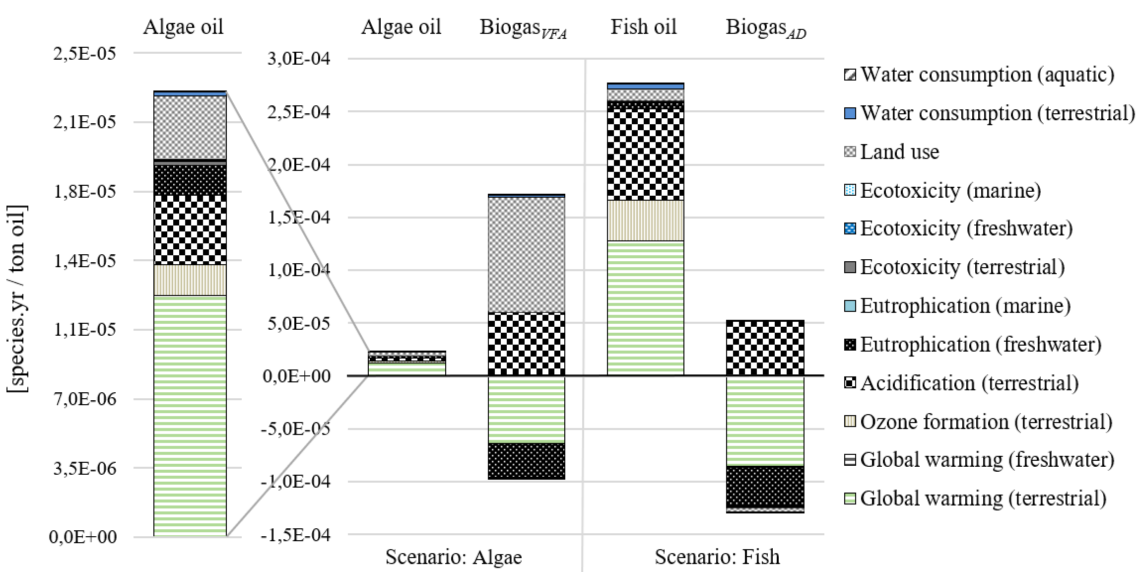


Figure 14: Ecosystem damage per ton oil, shown for each midpoint impact category.

The results show that the Algae scenario in total mitigate global warming and freshwater eutrophication while contributing to acidification and land use. Only eutrophication was mitigated for the Fish scenario while global warming, acidification and ozone formation infered increased damage, see Figure 28 in Appendix F.

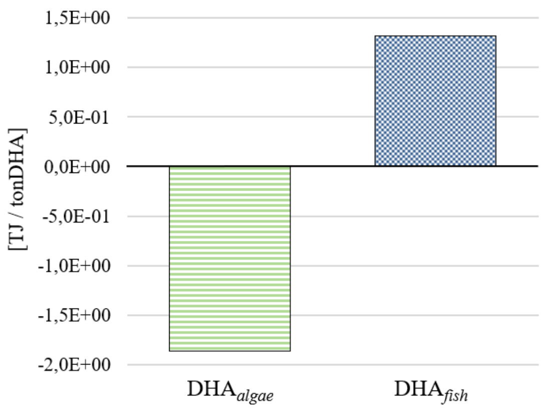
4.2 Environmental impact: DHA from algae and fish

This section provides numerical results obtained for environmental impact per tonDHA. DHA_{algae} represent environmental impact per tonDHA produced by the Algae scenario and DHA_{fish} represent impact per tonDHA from the Fish scenario. Environmental impact for DHA_{algae} and DHA_{fish} with respect to $ADP_{fossil fuels}$, GWP_{100} and Ecosystem damage are shown in Table 7.

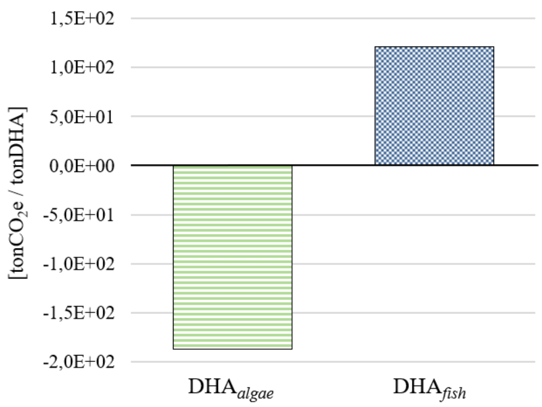
Table 7: Environmental impact per ton oil and per tonDHA.

Assessed impact	**Unit**	**DHA_{algae}**	**DHA_{fish}**
$ADP_{fossil fuels}$	TJ / tonDHA	-1,9E+00	1,3E+00
GWP_{100}	$tonCO_2e$ / tonDHA	-1,9E+02	1,2E+02
Ecosystem damage	species.yr / tonDHA	9,7E-04	1,7E-03
DHA content	%	10	12

The results show that environmental impact for DHA_{fish} was positive with respect to all assessed impact categories, while DHA_{algae} showed a negative impact with respect to $ADP_{fossil fuesls}$ and GWP_{100}. For every tonDHA produced from Algae scenario global warming was mitigated and fossil fuel use reduced, see Figure 15.



(a) Abiotic resource depletion



(b) Global warming potential

Figure 15: Environmental impact with respect to $ADP_{fossil fuels}$ and GWP_{100}. The results show impact for the Algae scenario and Fish scenario, expressed per tonDHA.

The result for Ecosystem damage show a positive value for DHA_{fish} and DHA_{algae}, which infer a loss of biodiversity for every produced unit of DHA. The Fish scenario infer about 37% higher environmental impact per tonDHA in comparison to DHA produced from the Algae scenario, see Figure 16.

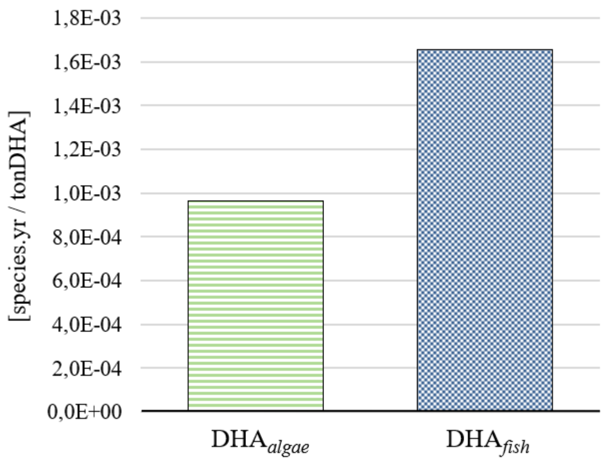


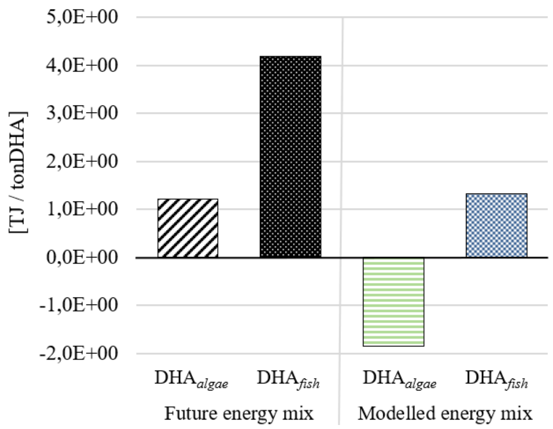
Figure 16: Ecosystem damage per tonDHA produced from the Algae and Fish scenario.

4.3 Sensitivity analysis

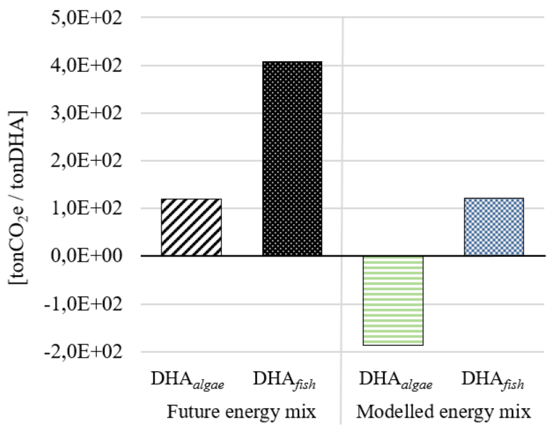
This section provide environmental impact results when a future energy mix, optimized VFA content and increased transport distance was simulated. Numerical results are presented in Appendix F.3.

4.3.1 Future energy mix

When biogas was modelled to substitute 100% renewable energy, the environmental impact for DHA_{algae} and DHA_{fish} increased. $ADP_{fossil fuels}$ and GWP_{100} for DHA_{algae} increased from a negative value to 1.2 TJ and 1.2E+02 $tonCO_2e$. Thus, when assuming a future energy mix, the environmental impact increased for each unit DHA produced. For DHA_{fish}, $ADP_{fossil fuels}$ and GWP_{100} increase with more than 60% to 4.2 TJ and 4.1E+02 $tonCO_2e$. The impact per tonDHA for DHA_{algae} when assuming future energy mix was similar to the impact from DHA_{fish} using current modelled energy mix, see Figure 17.



(a) Abiotic resource depletion



(b) Global warming potential

Figure 17: Environmental impact per tonDHA assuming 100% renewable energy.

The environmental impact with respect to Ecosystem damage shows that DHA_{algae} increase with 54% and DHA_{fish} with 49%, see Figure 18. DHA_{algae} increase to 2.1E-03 species.yr and DHA_{fish} to 2.7E-03 species.yr.

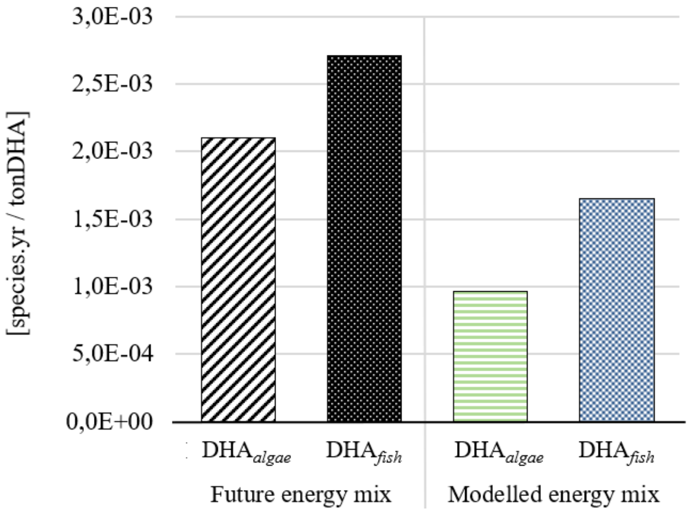
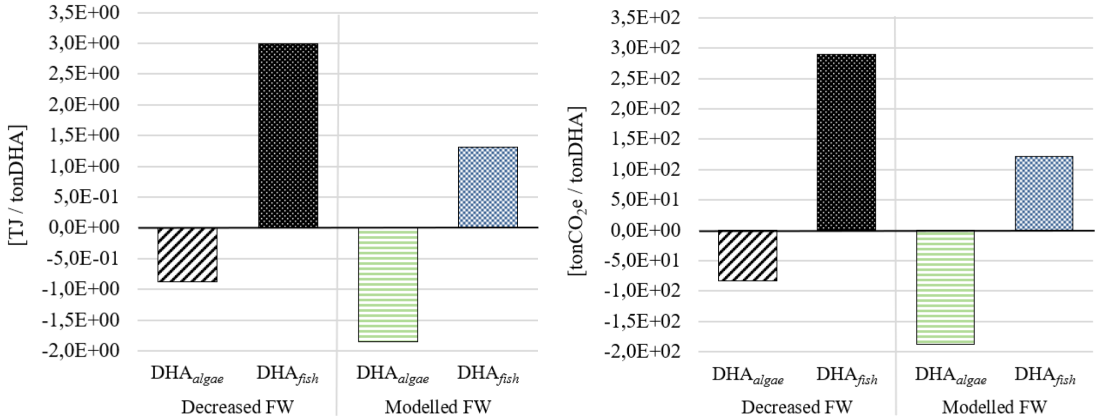


Figure 18: Ecosystem damage per tonDHA assuming 100% renewable energy.

4.3.2 Optimized VFA content

This section provide environmental impact when a dark fermentation process with optimized VFA production was simulated. The result shows that when less food waste was required, the environmental impact for DHA_{algae} and DHA_{fish} increase for all assessed impact categories. For DHA_{algae}, $ADP_{fossilfuels}$ and GWP_{100} increase with around 54% respectively while impact from DHA_{fish} with respect to $ADP_{fossilfuels}$ and GWP_{100} increase with about 56%, see Figure 19.



(a) Abiotic resource depletion

(b) Global warming potential

Figure 19: Environmental impact per tonDHA with optimized VFA content.

Environmental impact with respect to Ecosystem damage increase when less food waste was required, which is illustrated in Figure 20. The result shows that impact from DHA_{algae} increase with 17% and the impact from DHA_{fish} with 21%.

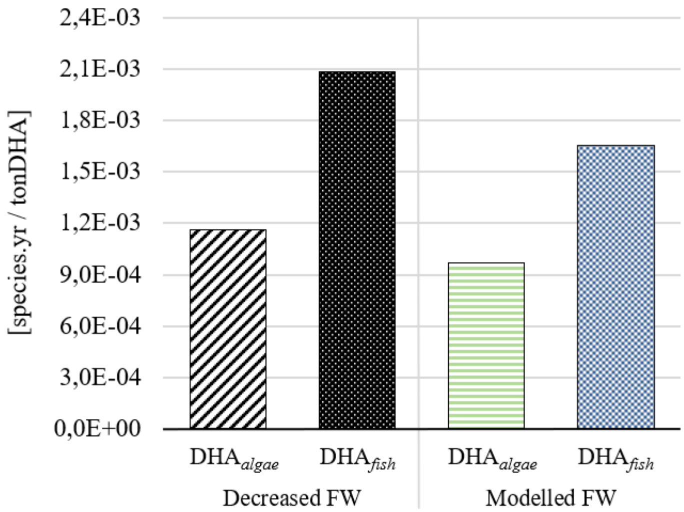
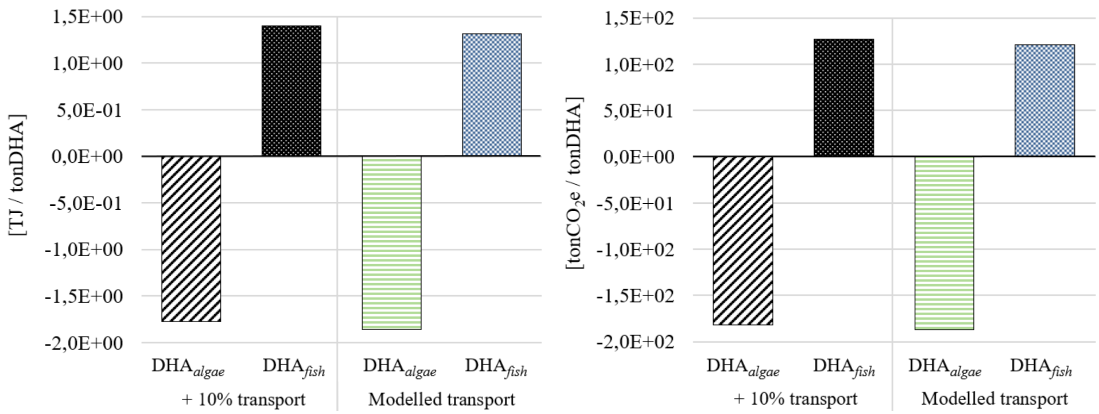


Figure 20: Ecosystem damage per tonDHA with optimized VFA content.

4.3.3 Increased transport

When the transport distance was increased, the result shows that environmental impact for ADP$_{fossil fuels}$, GWP$_{100}$ and Ecosystem damage increase. Impact from DHA$_{algae}$ with respect to all assessed impact categories increased with about 4% while ADP$_{fossil fuels}$ and GWP$_{100}$ for DHA$_{fish}$ increased with about 5% and Ecosystem damage increased with 2%. The results are shown in Figure 21 and 22.



(a) Abiotic resource depletion

(b) Global warming potential

Figure 21: Environmental impact per tonDHA with increased transport.

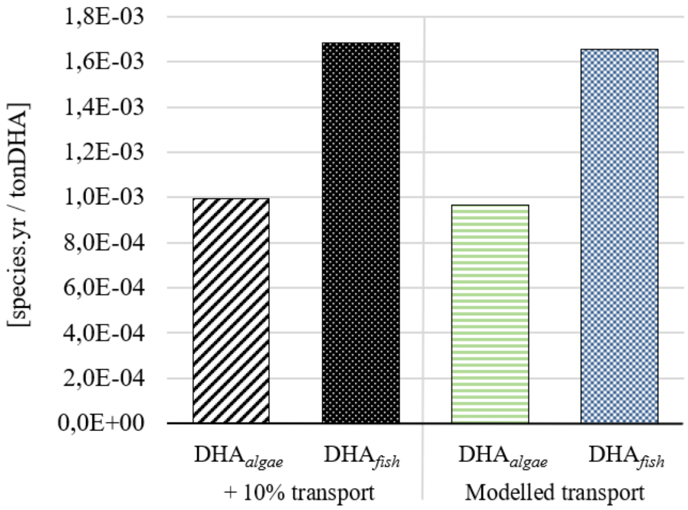


Figure 22: Ecosystem damage per tonDHA with increased transport distances.

5 Discussion

5.1 Environmental impact

5.1.1 Algae scenario and Fish scenario

The highest environmental impact from algae oil with respect to $ADP_{fossil fuels}$ and GWP_{100} was due to cultivation and harvest, see Figure 11. This was likely caused by required electricity and heat used for temperature control and mixing, sourced from German energy mix highly dependent on fossil fuels (IEA 2018). The results support previous research by Rösch et al (2018), who found that the cultivation and harvest process yields the highest environmental impact. In this thesis, the cultivation and harvest also infer the highest Ecosystem damage, where global warming, acidification and land use were the main contributors, see Figure 14. These damage indicators could be related to the use of fossil derived energy, but also required processing units. Rösch et al (2018) suggest that global warming constitutes the main environmental impact for algae production, which was supported by the results in this thesis. Another important factor for Ecosystem damage caused by algae oil and fish oil was the formation of terrestrial ozone, see Figure 14. The cause could be fossil NOx emissions, which via photochemical reactions in the atmosphere are converted into ozone (Bernes 2016). The result suggest that algae oil infer higher contribution to ozone formation than fish oil, which can be linked to the dataset used to describe harvest and cultivation processing (see Table 1).

The reduction fishery process, see Figure 11, contribute to the highest $ADP_{fossil fuels}$ impact which suggest a highly dependency on fossil fuels. According to Troell et al (2004) reduction fisheries require large amount of energy to reduce fresh fish to fish oil. Since this process uses multiple machinery to prepare, cook and dry fish, a high value for ADP was expected. Similar results were obtained for fish oil with respect to GWP_{100}, see Figure 12. Since the fish oil process does not contribute to methane emissions, GWP will include CO_2 and N_2O emissions primarily caused by combustion of fossil fuels. Therefore, ADP and GWP can have similar impact. However, in a study by Silva et al (2018) the $ADP_{fossil fuels}$ for fish oil produced from Peruvian anchovy was ten times lower and

GWP_{100} ten times higher than assessed in this thesis. Their result further suggest that the fishing would contribute to 42% of ADP instead of 4% as the result in this thesis show. Ziegler et al (2016) also suggest that the fishing process often dominates global warming due to the dependency of fossil fuels. The result for ADP and GWP assessed in this study could thereby not support previous research. The use of input data determines the result of LCA and could explain the difference in numerical results. Silva et al (2018) used SimaPro 8.3 and Ecoinvent 3.3, that did not contain updated fishing and Peruvian site locations. Accordingly, their LCI used input and output data from literature instead of pre-allocated data which likely provide a more comprehensive model.

An interesting result was the considerable impact biogas had on total environmental impact for algae oil and fish oil. When biogas was included via system expansion, see Table 6, the environmental impact for algae oil increased biodiversity for every ton oil produced, while the negative impact from fish oil was reduced. Moreover, environmental impact from dark fermentation was similar to anaerobic digestion for all assessed impact categories despite a 13% biogas loss due to VFA extraction. The largest factor for Ecosystem damage was land use and acidification, see Figure 14, which are both likely to be caused by the dataset used to describe anaerobic digestion and dark fermentation. When simulating a large scale production to assess VFA separation, additional buildings and energy are required in comparison to traditional biogas production. The result from this thesis shows that the main difference between biogas produced from dark fermentation and biogas produced from anaerobic digestion, was the VFA separation process. This process requires additional energy and land use, which Tampio et al (2018) also concluded.

5.1.2 DHA from algae and fish

The environmental impact per tonDHA shows that for every assessed impact category, microalgae fed VFA from food waste generate lower impact in comparison to fish oil, see Figure 15 and 16. These results are assessed using 10% DHA content for algae oil and 12% for fish oil, as shown in Table 7. Although, research suggest that cultivation and harvest methods, as well as feedstock, could increase the DHA content in algae oil (Chalima et al 2020; Mendes et al 2009). Meanwhile, the future DHA synthesis in marine ecosystems is estimated to decrease due to global warming (Colombo et al 2019). This would potentially decrease the natural DHA content in wild Peruvian anchovy, which in turn would increase the environmental impact per tonDHA produced. The Algae scenario uses about 150 g marine raw material while the Fish scenario require 22.2 ton, see Figure 9 and 10, which indicates that the Algae scenario could be less sensitive to a change in DHA synthesis.

Even though more oil was required and less biogas was produced per tonDHA from the Algae scenario in comparison to Fish scenario, the result in Figure 16 show that Ecosystem damage was about 37% lower for DHA_{algae} than DHA_{fish}. Additionally, even without the inclusion of $Biogas_{VFA}$ the Algae scenario was better from an environmental point of view, since fish oil generated ten times the impact for every tonDHA produced (see Appendix F.1 and Appendix F.2). Thus, the result show that dark fermentation of food waste for VFA production could support sustainable development for future DHA production and waste-to-value techniques.

5.1.3 Sensitivity analysis

The result from the sensitivity analysis shows that environmental impact from DHA produced from microalgae and fish oil increase when 100% renewable energy, optimized VFA content and 10% increased transports are simulated. The biggest difference occur when biogas was assumed to substitute renewable energy, see Figure 17 and 18, which suggests that the benefit from electricity and heat production from biogas decrease when the energi mix was renewable. This constitutes an important conclusion for future development, since the aim according to WWF (2012) is to reach 100% renewable energy by 2050. According to the *The waste hierarchy*, the re-use of food waste should be consistent with the most efficient use (EPA 2017). The sensitivity result suggest that a different use than biogas production for electricity and heat could be more efficient in the future. Furthermore, important to note is that these results show impact assessed for German energy mix which means that countries with higher share of renewable energy could obtain environmental impact results similar to the result of this sensitivity analysis. Interestingly, the impact for DHA_{algae} assuming future energy mix was similar to the impact from DHA_{fish} using current energy mix, see Figure 17. This result can be important from a future development perspective.

The second largest difference occurs when an optimized VFA content was simulated, see Figure 19 and 20. When less food waste was required to produce VFA, the amount of produced biogas decreased. This was likely the main cause for increased environmental impact since biogas used to substitute fossil energy mitigate global warming and decrease dependency on fossil fuels (World Biogas Association 2018). With respect to global warming and use of fossil fuels both scenarios increased with around 55% but DHA_{algae} maintained a mitigating impact while DHA_{fish} increased impact, see Figure 19. The result show that when VFA content was optimized, the Ecosystem damage increase with 21% and 17% for DHA_{fish} and DHA_{algae} respectively. This suggest that the Fish scenario was more sensitive for changes in biogas production in comparison to Algae scenario, likely due to the dependency on fossil fuels.

The final sensitivity analysis assessed transport distance, where only a slight increase in environmental impact was shown in Figure 21 and 22. The result show that when a 10% increased distance was modelled, the environmental impact for DHA_{fish} increase with 5% and 2% for DHA_{algae}. This result suggest that the aspect of transport distance was not sensitive to assumptions in the developed model. However, vehicles used were assumed to meet the European emission standard Euro VI which limit their emissions (European commission 2019b). This was likely the cause for low sensitivity to increased transport. If a lower euro standard was assumed, the emissions would likely increase and the model would accordingly be more sensitive to increased transport distance.

5.2 Uncertainties

5.2.1 Available data and modelling

The LCI parameters used in this thesis have impacted the results and was thereby a source of uncertainty. For instance, Ecoinvent 3.5 contain data for fishing activities and Peruvian site locations but datasets for algae aquaculture are currently not available (Avadí et al 2019). This infer a parameter uncertainty for LCI, that could potentially be mitigated by using primary data for foreground processes (Rosenbaum et al 2018). At present, most available data for algae oil production is developed for algae fuel production (Smetana et al 2017). The LCI data for algae oil production, see Table 1, was primarily sourced from Hosseinzadeh-Bandbafha et al (2019) describing algae fuel production. Even though the data used to describe the algae oil process could be considered less tailored to the process in comparison to production of fish oil, the production process is according to Rösch et al (2018) similar algae oil used for food and fuel. An increased pool of available LCI and data for aquaculture would enable a more representative assessment of DHA produced from algae.

Fishing LCI used pre-allocated data from Ecoinvent that only included impact caused by capture with steel purse seiners. The result in Figure 11 show that fishing only account for 4% of ADP for fish oil, which is 38% lower than assessed by Silva et al (2018). This illustrate that the LCI used affect the result, as suggested by Rosenbaum et al (2018). This was important to highlight in order to enable improvements in future studies. Furthermore, the dark fermentation was assumed to be identical to anaerobic digestion in this thesis, see Figure 8, and was modelled using the same dataset to assess environmental impact. In practice, the production processes differ and dark fermentation yields a higher VFA content in comparison to anaerobic digestion (Bharathiraja et al 2016). Therefore, a more representative assessment could be obtained using LCI data for dark fermentation.

5.2.2 Effects on biodiversity

Since available LCIA methods only include indirect effects and three of five identified drivers for biodiversity loss (Cenna et al 2018; Woods et al 2016), a considerable uncertainty was linked to the numerical value for Ecosystem damage. As suggested by previous research (Hélias et al 2018; Winter 2017), the direct impact on biodiversity caused by removal of aquatic biomass would infer a higher environmental impact than assessed in this thesis. Therefore, the Ecosystem damage from algae oil and fish oil are probably underestimated in this thesis. Since the algae production was less dependent on marine raw material, see Figure 9 and 10, the Ecosystem damage caused by algae removal can be considered less uncertain in comparison to anchovy. Although, removal of primary producers could cause damage to higher tropic levels which should be considered. Moreover, the data used to assess fish oil represent sustainable fishing while CIWF (2019) and FAO (2018) highlight that unsustainable fishing is still common. If the anchovy used for fish oil was sourced from unsustainable fishing, the environmental impact would be much higher than assessed in this thesis.

5.3 Future outlook

5.3.1 Global warming and sustainable development

One of the most urgent global challenges of today is to reach the SDG goals, including actions towards mitigation of climate change, reduced dependency on fossil fuels and promotion of biodiversity (United Nations 2019; Center for Biological Diversity 2020; Gutiérrez et al 2016). Since the natural DHA synthesis by marine microalgae is estimated to decrease with 58% until 2100 due to global warming (Colombo et al 2019), it becomes increasingly important to develop alternative ways to produce the essential fatty acid.

Important to note is that some benefits attributed to algae oil in this study are likely amplified when compared to fish oil, as fish oil can be considered unsustainable (CIWF 2019). Identifying one solution with a lower environmental impact in comparison to the commercial production method, does not necessarily mean that the suggested approach is the best solution. Alternative production methods should be carefully assessed to prevent substituting fish oil with another potentially unsustainable source. Therefore, different DHA producing methods should be evaluated in addition to comparing their impact with fish oil.

However, algae oil offer an important advantage in comparison to fish oil since feedstock, production methods and type of high-added value products obtained can be adjusted to a wide range of applications. Additionally, the Algae scenario in Figure 9 was less dependent on marine raw materials in comparison to the Fish scenario in Figure 10. Thus, DHA produced from algae oil would likely be less sensitive to extreme weather and climate change, which could pose as a considerable advantage as global warming is estimated to increase (IPCC 2018; Bernes 2016). As this thesis shows, algae oil production in combination with food waste-to-value techniques and energy production can contribute to the SDG *climate change action*, *clean energy production* and *conservation of biodiversity*. DHA production from algae oil thereby hold a promising potential for sustainable development within aquaculture with waste-to-value techniques (Chalima et al 2017).

5.3.2 Biodiversity in LCA

Even though LCA is well developed for resource use and emissions, the impact on biotic and living resources is still incomplete (Winter et al 2017; Woods et al 2016). 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. The environmental impact for Ecosystem damage assessed in this thesis include effects on biodiversity established in current LCIA methods, but would be more complete if direct effects could also be included. As suggested by Klöpffer & Grahl (2016b), LCIA methods correspond to current knowledge and modelling ability. One could therefore argue that the most important future research area is to reduce knowledge gaps and develop LCIA methods that, preferably, covers all five drivers for biodiversity loss. This would enable a more robust and complete environmental assessment. To achieve this, further research and method development is needed. Crenna et al (2018) propose a research agenda to achieve a comprehensive accounting and characterization biotic resources, which could serve as a foundation for further research. Additionally, Woods et al (2016) identify important aspects to consider when incorporating marine biodiversity drivers in LCA.

One method that includes all five drivers and could be applied to other LCIA methods is the Product Biodiversity Footprint suggested by Asselin et al (2020). According to Asselin (22 May 2020) the aim is to also include marine biodiversity. By using established impact methods, biodiversity can be assessed in a comparable and transparent way, which is in line with the ISO standard and LCA framework. However, since method development is complex and time consuming, it would presumably be more efficient to gradually develop and implement one factor at a time. For instance, characterization factors for direct effects on marine biotic resources suggested by Hélias et al (2018) could be implemented. An LCA that better describe environmental impact on biodiversity would be a powerful tool to promote sustainable development.

5.3.3 Waste-to-value

The aim with a food waste-to-value approach is to create circular loops where no resources are wasted and to re-use food waste in the most efficient way (FAO 2017; EPA 2017). Therefore, it is important to continuously develop and assess waste-to-value techniques that re-use food waste to produce different high value products, such as VFA. Since VFA can be used as feed and energy (Chalima et al 2020; Khan et al 2016), it could be considered more valuable and versatile in comparison to methane primarily used for energy. Sustainable development within waste-to-value techniques should aim to produce high value products (EPA 2017), which further emphasize the importance of researching VFA extraction and re-use from dark fermentation.

The sensitivity analysis for future energy mix, see Figure 17 and 18, suggest that biogas for electricity and heat will be less valuable in the future. This indicates that it could be beneficial to consider a different method for food waste reuse. Potentially, it could be more efficient to upgrade biogas produced after VFA is extracted to biomethane used as vehicle fuel (World Biogas Association 2019; Bharathiraja et al 2016).

Another potential food waste-to-value solution could be to produce a different energy carrier from the remaining food waste when VFA has been extracted. According to Tampio et al (2018), H_2 can be considered a more valuable resource than CH_4 and could therefore contribute to a more efficient reuse of food waste. This valorization method could be assessed using a similar system as suggested in this thesis, but using a dark fermentation process like the one suggested by Chalima et al (2020) where the remaining food waste is used for H_2 production instead of biogas.

5.3.4 EU legislation

The animal by-product legislation infer that DHA produced from algae fed VFA extracted from food waste, is at present likely prohibited. Hence, the results of this thesis can only be implemented in real systems if derogation are granted from the EU or if the legislation is changed. A suggestion is that a risk assessment is carried out in order to identify relevant risks and hazards when using DHA oil produced from food waste in food production. If the risk assessment show a negligible risk of contamination, one can argue that EU legislation need to change. An update in EU legislation would further enable waste-to-value techniques in the future and facilitate a major global resource challenge.

Potentially, vegetable waste from agriculture could be used instead of food waste to produce VFA. This could enable the suggested approach in this thesis, shown in Figure 8, to be implemented in current production systems. However, using only vegetable waste could change the nutrient and energy composition of the food waste, which might alter the amount of VFA produced in dark fermentation (Strazzera et al 2018).

5.3.5 Aquaculture

The aquaculture sector will likely continue to grow (Avadí et al 2019; FAO 2018), leading to a high demand for sustainable production methods to maintain natural ecosystems while providing the global population with nutritious food. Most DHA consumed by humans is today derived from fish oil, and the demand for DHA will likely increase with global population (FAO 2018; FAO 2019). Algae oil could therefore bridge a future gap between supply and demand. Since DHA produced from the Algae scenario infers lower environmental impact with respect to global warming, use of fossil fuels and effects on biodiversity, one could argue that fish oil in fish feed should be replaced with algae oil. However, its important to note that fish oil is rich in both DHA and EPA while algae oil from C.Cohnii is dominated by DHA. Substituting directly could thereby infer altered nutritional value in fish feed, as suggested by Sprague et al (2015).

A potential solution could be to use a different algae species, for instance *Schizochytrium*, that produce EPA and DHA content similar to fish oil (Winwood 2013). Another possibility could be to use DHA from C.Cohnii assessed in this thesis for other food supplements, provided a reassuring risk assessment and change in EU legislation. Future research could also include a time aspect in order to relate impact to global demand, for instance by assessing environmental impact for tonDHA required per year. A time aspect could be assessed in relation to the estimated demand for nutritious food due to increased global population.

Since algae aquaculture today constitutes of less than 1% of total aquaculture (FAO 2018), the production methods and energy efficiency can be considered to be in early development stages when compared to fish oil production that has been optimized and streamlined for decades. As suggested by Taleman et al (2013) the algae production could be improved via nutrient recycling, efficient energy use and upscaling to larger production. If future production methods can be optimized for algae aquaculture, the environmental impact is likely to decrease even more, while enabling improved conditions for SDG fulfillment.

5.4 Recommendations for future studies

The model developed in this thesis could be improved by adapting LCI for pre-cultivation, fishing and dark fermentation. The pre-cultivation of C.Cohnii could be modelled with inputs similar to LCI for starting culture, cultivation and harvest in Table 1. This would ensure a better representation of heterotrophic cultivation of microalgae. Additionally, the fishing process in Table 3 could be modelled with similar inputs as suggested by Silva et al (2018) to address more effects of fishing. Finally, LCI for dark fermentation should be used instead of LCI in Table 2 which describes anaerobic digestion.

Since direct effects on biodiversity can not be assessed with established LCIA methods, future studies would likely 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 primarily be included in studies with a marine biodiversity focus. This could potentially be accomplished by implementing characterization factors for marine biotic resource depletion (Hélias et al 2018), overfishing (Emanuelsson et al 2014) or invasive species (Hanafiah et al 2013). By including these characterization factors in combination with for instance the ReCiPe Endpoint method, a more complete assessment for biodiversity loss could be possible.

6 Conclusion

This thesis assessed environmental impact from DHA produced by microalgae using VFA extracted from dark fermentation of food waste. The remaining biomass was used for biogas production used for electricity and heat. The impact per tonDHA produced from algae was -1.9E+02 tonCO_2e, -1.9 TJ and 9.7E-04 species.yr. DHA produced from algae caused 37% lower Ecosystem damage in comparison to DHA from fish, even though established LCIA methods only assess indirect effects on biodiversity. This thesis showed that algae oil infer lower environmental impact per ton oil in comparison to fish oil with respect to global warming, fossil energy use and effects on biodiversity. Biogas used to produce electricity and heat reduce dependency on fossil energy, which decrease the total environmental impact caused from production of algae oil and fish oil. The sensitivity analysis shows that DHA produced by microalgae infered lower environmental impact in comparison to fish oil even when 100% renewable energy, optimized VFA production and increased transports were simulated. Therefore, DHA produced by microalgae using VFA from dark fermentation of food waste could mitigate loss of biodiversity, while reducing dependency on marine raw materials and fossil fuels. This in turn could support sustainable development by meeting current need of DHA without compromising the natures ability to produce DHA in the future.

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Appendices

A Inventory analysis data

A.1 Algae starting culture

This subsection provides a model calculation for C.Cohnii required per ton algae oil when simulating a large scale production. See Table 8 for reference data used.

Table 8: Literature values for algae inputs used for LCI calculations.

Input	Amount	Unit	Reference	Input	Amount	Unit
VFA	200	kg / batch	Limonne & Junne (5 February 2020)	VFA	2,00E+02	kg / batch
C. Cohnii	1	%	Hosseinzadeh et al (2019)	C. Cohnii	1,80E+01	kg / batch
Yeast	2	g / L	Chalima et al (2020)	Yeast	3,60E+00	kg / batch
Reef salt	25	g / L	Chalima et al (2020)	Reef salt	4,50E+01	kg / batch
Molasses	9	g / L	Diao et al (2018)	Molasses	1,62E+01	kg / batch
Water	1480	L		Water	1,48E+03	kg / batch
Batch size	1800	L				

Batches / year	% DHA	kg algae / year	Reference
7,40E+02	1,00E-01	3,70E+05	Limonne & Junne (5 February 2020)

Data for molasses was collected from Diao et al (2018), assuming that molasses could be used instead of glucose. According to Hosseinzadeh-Bandbafha et al (2019) the amount of algae required for starting culture is less than 1% of batch size. This thesis assume a batch size of 1800 L and a required algae concentration of 0.1%, the total amount of C.Cohnii needed (kg/year) was calculated in equation (1).

$$\text{C.Cohnii}_{year} = 18\frac{kg}{batch} \times 740\frac{batches}{year} \approx 13300 \tag{1}$$

The amount of C.Cohnii required per unit algae was calculated by dividing the amount needed per year with the amount of algae obtained per year. To translate this value into ton C.Cohnii needed (ton/ton algae oil), the value was divided by 0.24 (assuming 24% oil), see equation (2).

$$\text{C.Cohnii} = \frac{13300}{370000} \times \frac{1}{0.24} = 0.15 \tag{2}$$

The same calculation principle was applied to all pre-cultivation inputs. Table 9 provides calculated amounts of inputs needed per ton algae oil. When assuming that 1 ton algae oil and 1.87 ton algae solids are obtained, the amount C.Cohnii needed was about 0.5%.

Table 9: Calculated LCI values used in Table 1 for algae oil production.

Input	kg / year	kg / kg algae	kg / kg algae oil	ton / ton algae oil
VFA	1,48E+05	4,00E-01	1,67E+00	1,67E+00
C. Cohnii	1,33E+04	3,60E-02	1,50E-01	1,50E-01
Yeast	2,66E+03	7,20E-03	3,00E-02	3,00E-02
Reef salt	3,33E+04	9,00E-02	3,75E-01	3,75E-01
Molasses	1,20E+04	3,24E-02	1,35E-01	1,35E-01
Water	1,10E+06	2,96E+00	1,23E+01	1,23E+01

A.2 C.Cohnii pre-cultivation

This subsection provides a summary Table 10 of the calculated LCI needed to pre-cultivate C.Cohnii used per ton algae oil. In this thesis, 10g wild algae is assumed to be required to grow 1 kg C.Cohnii. Data was collected from Hosseinzadeh-Bandbafha et al (2019) and multiplied by 0.015 ton to obtain the required wild C.Cohnii per ton algae oil. The CO_2 emissions were set to 0.1 kg / kg algae as suggested by Silva et al (2019).

Table 10: Summary table for LCI data required to grow 0.015 ton C.Cohnii.

Input / kg dry algae	Amount	Unit	Input / ton algae oil	Amount	Unit
Wild C.Cohnii	1,00E-02	kg	Wild C.Cohnii	1,50E+00	kg
Nitrogen fertilizer	2,60E-01	kg	Nitrogen fertilizer	3,90E+01	kg
Phosphorus fertilizer	4,50E-02	kg	Phosphorus fertilizer	6,75E+00	kg
Freshwater	1,67E+00	m3	Freshwater	2,51E+02	m3
Electricity	8,50E+01	kWh	Electricity	1,28E+04	kWh
Flue gas	-1,81E+02	kg	Flue gas	-2,72E+04	kg
Output / kg dry algae	**Amount**	**Unit**	**Output / ton algae oil**	**Amount**	**Unit**
CO_2 to air	1,79E+02	kg	CO_2 to air	2,69E+04	kg
C.Cohnii	1,00E+00	kg	C.Cohnii	1,50E+02	kg

A.3 Plastic and metal reject

From the collected food waste, a total of 2.88 ton is rejected (3% of total collected food waste), of which 50% is metal and 50% is plastic. The plastic reject was assumed to be re-used in municipal waste incineration, while the metal reject was re-cycled. The energy in plastic was assumed to be 4.64E+07 J/kg (Hamman 2010) and 1.44 ton plastic reject was generated per ton algae oil. The amount of energy (MJ) needed to process the plastic was calculated based on the energy in plastic, see equation (3).

$$\text{Plastic reject} = 1.44 \times 1000 \times 4.64E+07 \approx 6.68E+04 \tag{3}$$

B Buswell equation and COD calculation

In this section, the theoretical loss of biogas due to VFA extraction is calculated via Chemical Oxygen Demand and the Buswell equation. These calculations use that the energy carrying compound in biogas is CH_4.

To illustrate the oxygen required (COD) for VFA, a model calculation for COD acetic acid is conducted. In AD the chemical decomposition of organic matter is preserved in CH_4 and CO_2. When biogas is produced from fully biodegradable organic substances in AD, the Buswell's equation can be used by balancing the chemical reaction according to equation (4).

$$C_aH_bO_c + (a - \tfrac{1}{4}b - \tfrac{1}{2}c)H_2O \rightarrow (\tfrac{1}{2}a + \tfrac{1}{8}b - \tfrac{1}{4}c)CH_4 + (\tfrac{1}{2}a - \tfrac{1}{8}b + \tfrac{1}{4}c)CO_2 \tag{4}$$

For acetic acid, with molecular formula $C_2H_4O_2$ and molecular weight 60 g/mol, the chemical reaction is balanced according to equation (5).

$$C_2H_4O_2 + 2O_2 \rightarrow 2CO_2 + 2CH_4 \tag{5}$$

In equation (5) it is stated that for each mol acetic acid, 2 moles oxygen is required. The molar weight of oxygen is 2×16 grams, hence 2×2×16 = 64 grams of oxygen is needed. The amount of O_2 (g) required for acetic acid was calculated according to equation (6).

$$\mathrm{O_2} = \tfrac{64}{60} = 1.07 \tag{6}$$

Assuming that 91% of 25 g VFA/ L food waste can be extracted, 1.67 ton VFA is needed and that acetic acid was 26%, the amount of acetic acid (ton / ton algae oil) was calculated according to equation (7).

$$\text{Acetic acid} = \tfrac{0.26}{0.91} \times 1.67 \approx 4.80\mathrm{E}-01 \tag{7}$$

The COD for acetic acid (ton COD) is obtained by multiplying the amount of acetic acid with required amount of O_2, see equation (8).

$$\mathrm{COD}_{aceticacid} = 4.80\mathrm{E}-01 \times 1.07 \approx 5.13\mathrm{E}-01 \tag{8}$$

The CH_4 loss (m^3) due to acidic acid extraction is obtained by multiplying the amount acetic acid with 0.35 L CH_4/ g COD (Nordberg, 15 May 2020). Using that 1 L is equal to 0.001 m^3, the loss was calculated according to equation 9.

$$\mathrm{CH_4} = 5.13\mathrm{E}-01 \times 0.35 \times 1000 \approx 1.80\mathrm{E}+02 \tag{9}$$

COD for propionic acid and butyric acid was collected from Walker et al (2010). The same calculations are done for all VFA components. Table 11 provide inputs, assumptions and calculated values for COD.

Table 11: Calculated COD and CH_4 loss for per ton algae oil.

g VFA / L FW	Unit	Acetic acid	Propionic acid	Butyric acid	Total	Reference
2,50E+01	%	26%	38%	27%	91%	Garcia et al (2018)
	g O_2 / g VFA	1,07	1,51	1,82	-	Walker et al (2010)
L CH_4 / g COD	**Unit / ton algae oil**	**Acetic acid**	**Propionic acid**	**Butyric acid**	**Total**	
0,35	%	29%	41%	30%	100%	
	ton	4,80E-01	6,92E-01	4,98E-01	1,67E+00	
	ton COD	5,13E-01	1,04E+00	9,07E-01	2,47E+00	
	m^3 CH_4	1,80E+02	3,66E+02	3,17E+02	8,63E+02	

Assuming 60% CH_4 content in biogas, the biogas loss (m^3) due to VFA extraction was calculated by adding the total CH_4 loss for all VFA components, and multiplying by 0.6, see equation (10).

$$\text{Biogas}_{loss} = (1.80\text{E}+02+3.66\text{E}+02+3.17\text{E}+02) \times 0.6 \approx 1.44\text{E}+03 \tag{10}$$

C System expansion: Avoided energy

This section provides energy calculations when biogas substitute German energy mix (based on data from 2014), used for the system expansion in SimaPro. Following calculations illustrate how the numerical values in Table 12 are obtained.

Table 12: Avoided energy due to Biogas_{VFA} production. Input to table 2.

	Amount	**Unit**	**Distribution**
Biogas slurry (DF)	Amount	ton	
Solds in slurry	9,49E+01	ton	
Biogas potential	1,16E+04	m3	60% biogas
Biogas loss	1,16E+04	m3	
Biogas total	2,32E+04	m3	
Avoided energy	5,49E+04	kWh	90% efficiency rate

According to SGC (2012), 204 m^3 biogas can be obtained from 1 ton sorted food waste, e.g. solid components in AD. Food waste and algae solids are assumed to have equivalent biogas potential, and 60% is assumed to be biogas while 40% is digestate. The potential biogas (m^3) is calculated according to equation (11).

$$\text{Biogas}_{potential} = 9.49\text{E}+01 \times 204 \times 0.6 \approx 1.16\text{E}+04 \tag{11}$$

The biogas loss due to VFA extraction is subtracted from $\text{Biogas}_{potential}$. In this thesis, it is assumed that 1 m^3 biogas generate 6 kWh, with an additional efficiency rate of 90% (Rutz et al 2015). The total amount of energy (kWh) avoided can be calculated according to equation (12).

$$\text{Energy}_{avoided} = (1.16\text{E}+04 - 1.44\text{E}+03) \times 6 \times 0.9 \approx 5.49\text{E}+04 \quad (12)$$

The amount of avoided energy for biogas production without VFA extraction is calculated the same way, and summarized in Table 13.

Table 13: Avoided energy due to Biogas_{AD} production. Input to table 4.

	Amount	**Unit**	**Distribution**
Biogas slurry (AD)	1,46E+02	ton	
Solds in slurry	9,30E+01	ton	
Biogas potential	1,14E+04	m3	60% biogas
Biogas total	1,14E+04	m3	
Avoided energy	6,15E+04	kWh	90% efficiency rate

D Ecoinvent datasets used in SimaPro modelling

This section provides a list of the Ecoinvent datasets used in SimaPro 9 (PhD licence). Table 14 specify original data generator, dataset name, geographic location and which process the dataset models. All datasets are collected from Ecoinvent 3.5 with the *Allocation, cut-off by classification* setting.

Table 14: Datasets used to model production of algae oil, fish oil and biogas.

Number	Data generator	Dataset name	Location	Used to model
1	Symeonidis, A.	*Market for marine fish*	GLO	Wild C.Cohnii
2	System	*Market for nitrogen fertiliser, as N*	GLO	N fertiliser
3	System	*Market for phosphate fertiliser, as P2O5*	GLO	P fertiliser
4	Valsasina, L.	*Market for transport, freight, lorry 3.5-7.5 metric ton, EURO4*	RER	Transport
5	PRé Consultants	*Water, unspecified natural origin*	DE	Fresh water
6	Treyer, K.	*Market for electricity, medium voltage*	DE	Electricity
7	System	*Market for liquid manure storage and processing facility*	GLO	Algae processing
8	FitzGerald, D.	*Market for SOx retained, in hard coal flue gas desulfurisation*	RER	Flue gas
9	PRé Consultants	*Carbon dioxide, unspecified*	n.a.	CO_2 to air
10	System	*Market for wastewater, average*	Europe without Switzerland	Wastewater
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	Treyer, K.	*Market for heat, district or industrial, other than natural gas*	Europe without Switzerland	Heat
15	System	*Market for municipal waste collection service by 21 metric ton lorry*	GLO	Transport
16	System	*Market for waste preparation facility*	GLO	FW processing
17	PRé Consultants	*Recycling of aluminium*	GLO	Metal reject
18	Treyer, K.	*Treatment of municipal solid waste, incineration*	DE	Plastic reject
19	Levova, T.	*Market for tap water*	Europe without Switzerland	Water
20	Symeonidis, A.	*Anaerobic digestion of manure*	CH	AD processing
21	Gnansounou, E.	*Rape oil mill operation*	Europe without Switzerland	VFA processing
22	Treyer, K.	*Heat and power co-generation, biogas, gas engine*	DE	Biogas processing
23	Avadi, A.	*Anchovy, capture by steel purse seiner and landing whole, fresh*	PE	Fishing anchovy
24	Avadi, A.	*Fishmeal and fish oil production, 63-65% protein*	PE	Reduction fishery
25	System	*Market for transport, freight, lorry 3.5-7.5 metric, EURO4*	RoW	Transport
26	System	*Market for transport, freight, sea, transoceanic ship with reefer, cooling*	GLO	Transport
27	Treyer, K.	*Electricity, high voltage, wind power, import from Germany*	CH	Wind power
28	Treyer, K.	*Electricity production, photovoltaic, 570kWp open ground installation, multi-Si*	DE	Photovoltaic

E SimaPro 9 model set-up

This section provides illustrations of SimaPro model set-up. The impact are set to illustrate Ecosystem damage, where a broader arrow indicate a more substantial impact. The cut-off is set to 1%.

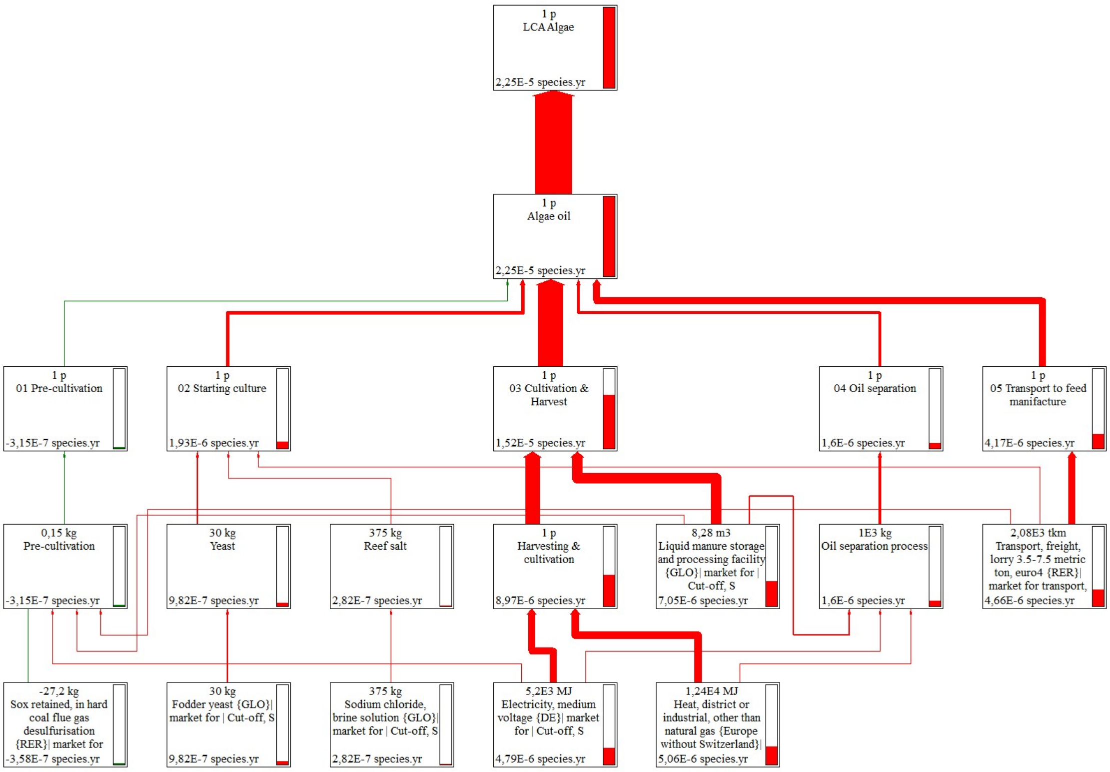

Figure 23: Illustration of model set-up for Algae oil production.

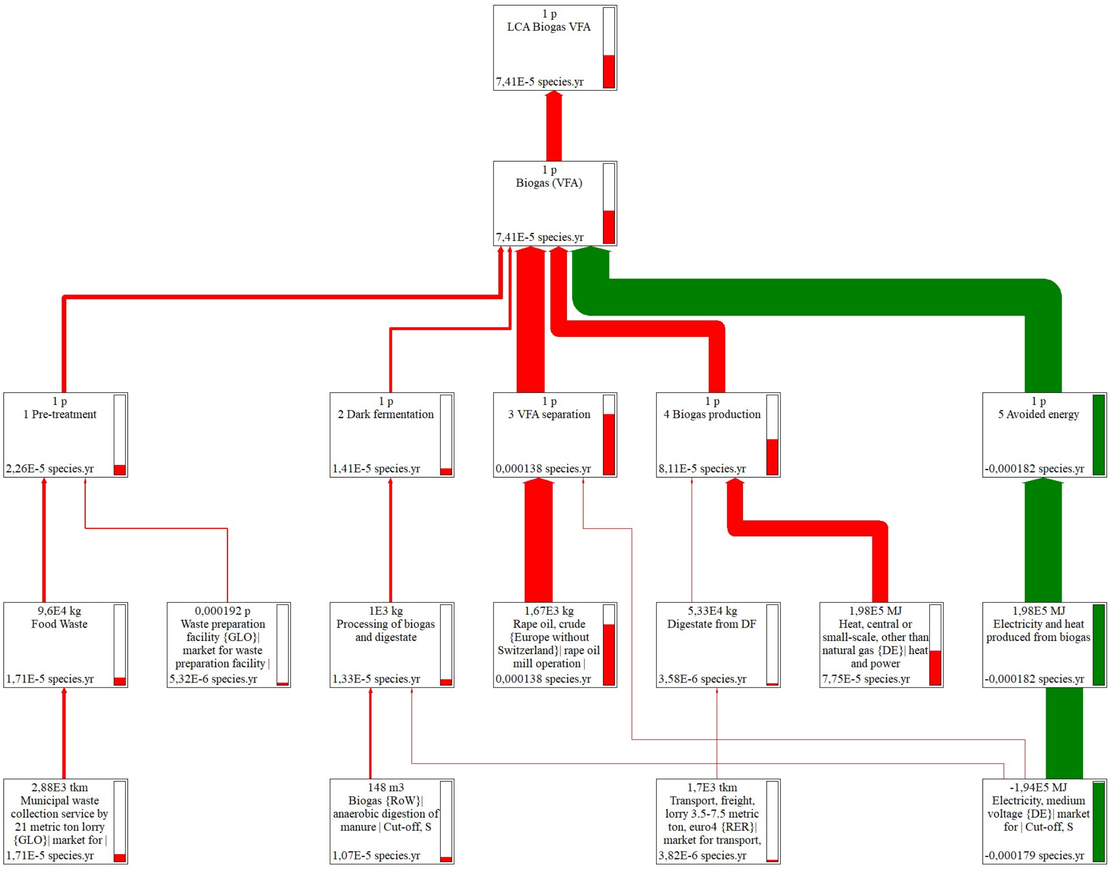

Figure 24: Illustration of model set-up for Biogas with VFA extraction (Biogas$_{VFA}$).

F Impact assessment

F.1 Global warming and fossil energy

This section provides supporting tables and figures for assessed environmental impact with respect to global warming and fossil energy use, expressed per ton oil. Table 15 show numerical results obtained from the SimaPro model.

Table 15: Environmental impact, expressed in TJ/ ton oil and tonCO_2e/ ton oil.

Scenario: Algae	**Algae oil**	**Pre-cultivation**	**Starting culture**	**Cultivation & Harvest**	**Oil separation**	**Transport**	**Total**
	$ADP_{fossil\ fuels}$	3,7E-06	2,8E-03	2,7E-02	2,8E-03	1,4E-02	4,6E-02
	GWP_{100a}	-1,6E-02	2,7E-01	2,8E+00	3,0E-01	9,4E-01	4,3E+00
	Biogas (VFA)	**Pre-treatment**	**Dark fermentation**	**VFA separation**	**Biogas production**	**Avoided energy**	**Total**
	$ADP_{fossil\ fuels}$	5,7E-02	5,9E-03	2,9E-02	1,8E-02	-3,4E-01	-2,3E-01
	GWP_{100a}	4,1E+00	8,4E-01	3,6E+00	3,3E+00	-3,5E+01	-2,3E+01
Scenario: Fish	**Fish oil**	**Fishing**	**Reduction fishery**	**Transport**	**Total**		
	$ADP_{fossil\ fuels}$	2,0E-02	4,0E-01	3,6E-02	4,6E-01		
	GWP_{100a}	1,4E+00	4,1E+01	2,5E+00	4,5E+01		
	Biogas	**Pre-treatment**	**Anaerobic digestion**	**Biogas production**	**Avoided energy**	**Total**	
	$ADP_{fossil\ fuels}$	5,7E-02	5,7E-03	1,9E-02	-3,8E-01	-3,0E-01	
	GWP_{100a}	4,1E+00	8,2E-01	3,6E+00	-3,9E+01	-3,1E+01	

The impact shown in table 15 are illustrated in Figure 25 and 26 to show each production process respective contribution to environmental impact.

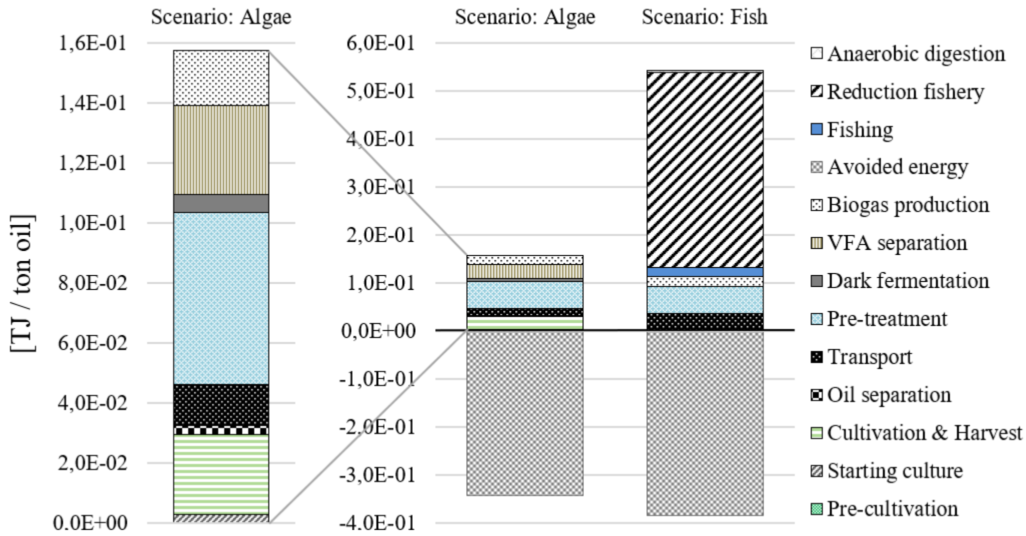

Figure 25: Total impact for the algae and fish scenario, with respect to $ADP_{fossil fuels}$.

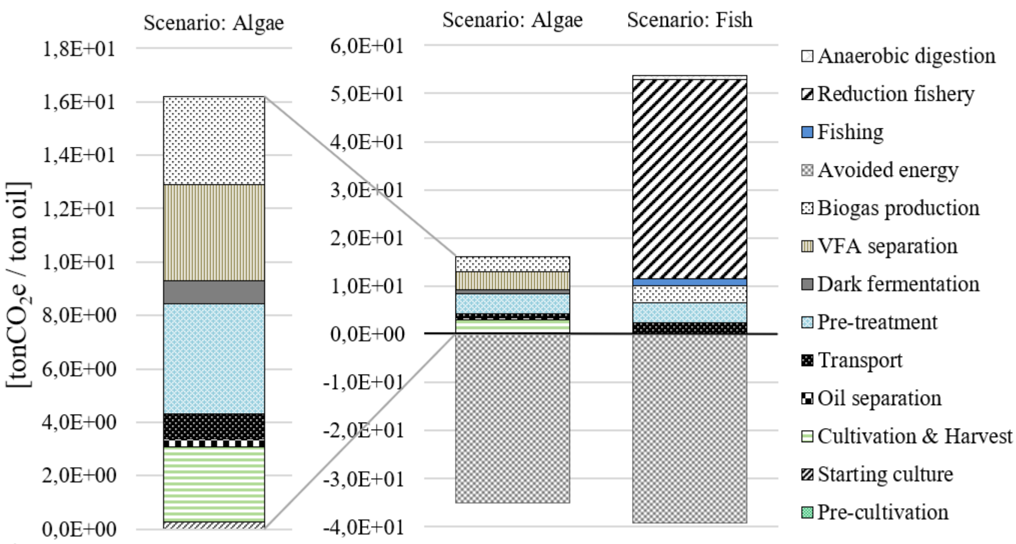

Figure 26: Total impact for the algae and fish scenario, with respect to GWP_{100}.

F.2 Biodiversity

This section provides supporting tables and figures for assessed environmental impact with respect to biodiversity, expressed per ton oil. Figure 27 illustrate the total Ecosystem damage for the Algae and Fish scenario, based on numerial results shown in Table 16 and Table 17.

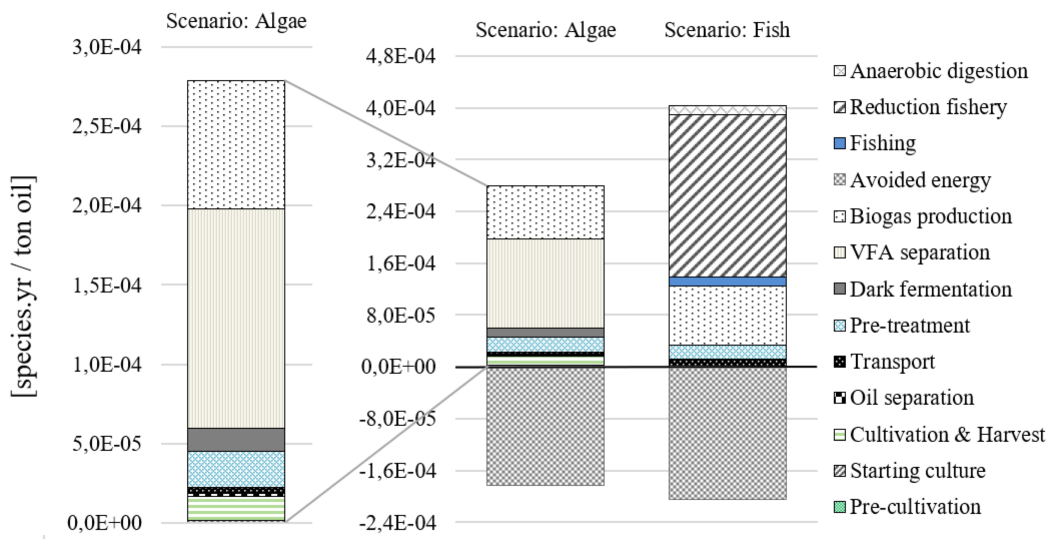

Figure 27: Total impact for the algae and fish scenario, with respect to Ecosystem damage.

Table 16 show results obtained from the SimaPro model (see Figure 23 and 24). The table show respective contribution to Ecosystem damage for each production process in the Algae scenario.

Table 16: Ecosystem damage for the Algae scenario, expressed in species.yr / ton oil.

Algae oil	**Pre-cultivation**	**Starting culture**	**Cultivation & Harvest**	**Oil separation**	**Transport**	**Total**
Global warming, Terrestrial ecosystems	-4,6E-08	7,7E-07	8,0E-06	8,5E-07	2,6E-06	1,2E-05
Global warming, Freshwater ecosystems	-1,3E-12	2,1E-11	2,2E-10	2,3E-11	7,2E-11	3,3E-10
Ozone formation, Terrestrial ecosystems	-1,1E-09	9,8E-08	9,0E-07	9,7E-08	4,9E-07	1,6E-06
Terrestrial acidification	-2,7E-07	2,6E-07	2,7E-06	2,5E-07	5,8E-07	3,5E-06
Freshwater eutrophication	6,9E-09	5,7E-08	1,2E-06	1,3E-07	7,1E-08	1,5E-06
Marine eutrophication	-1,4E-11	2,1E-10	3,0E-10	2,1E-11	1,3E-11	5,4E-10
Terrestrial ecotoxicity	-1,3E-10	2,1E-08	8,0E-08	9,6E-09	9,2E-08	2,0E-07
Freshwater ecotoxicity	-3,9E-10	7,8E-09	5,6E-08	6,7E-09	1,6E-08	8,6E-08
Marine ecotoxicity	-7,9E-11	1,7E-09	1,2E-08	1,4E-09	3,7E-09	1,9E-08
Land use	-7,5E-10	6,7E-07	2,1E-06	2,1E-07	2,4E-07	3,2E-06
Water consumption, Terrestrial ecosystem	-5,1E-09	4,1E-08	1,0E-07	3,1E-08	3,2E-08	2,0E-07
Water consumption, Aquatic ecosystems	-2,5E-13	5,3E-12	5,0E-12	1,5E-12	1,7E-12	1,3E-11
Ecosystem damage (Algae oil)	-3,2E-07	1,9E-06	1,5E-05	1,6E-06	4,2E-06	2,3E-05

Biogas (VFA)	**Pre-treatment**	**DF**	**VFA separation**	**Biogas production**	**Avoided energy**	**Total**
Global warming, Terrestrial ecosystems	1,2E-05	2,5E-06	1,1E-05	1,0E-05	-9,9E-05	-6,4E-05
Global warming, Freshwater ecosystems	3,2E-10	6,8E-11	2,9E-10	2,8E-10	-2,7E-09	-1,7E-09
Ozone formation, Terrestrial ecosystems	3,7E-06	9,6E-08	1,8E-06	7,1E-07	-4,9E-06	1,4E-06
Terrestrial acidification	3,5E-06	1,0E-05	1,1E-05	6,9E-05	-3,5E-05	5,9E-05
Freshwater eutrophication	4,3E-07	5,0E-07	9,0E-07	2,6E-07	-3,4E-05	-3,2E-05
Marine eutrophication	7,4E-11	8,2E-11	2,0E-08	4,3E-11	-5,5E-09	1,5E-08
Terrestrial ecotoxicity	1,8E-07	5,8E-09	1,0E-07	9,6E-08	-1,9E-07	1,9E-07
Freshwater ecotoxicity	7,4E-08	1,7E-08	5,5E-08	2,7E-08	-1,0E-06	-8,8E-07
Marine ecotoxicity	1,7E-08	3,4E-09	1,2E-08	6,0E-09	-2,2E-07	-1,8E-07
Land use	3,0E-06	1,6E-07	1,1E-04	6,3E-07	-6,9E-06	1,1E-04
Water consumption, Terrestrial ecosystem	1,2E-07	7,5E-07	2,1E-06	4,5E-08	-1,6E-06	1,4E-06
Water consumption, Aquatic ecosystems	6,2E-12	3,3E-11	4,4E-10	3,1E-12	-6,5E-11	4,1E-10
Ecosystem damage (Biogas VFA)	2,3E-05	1,4E-05	1,4E-04	8,1E-05	-1,8E-04	7,4E-05

Table 17 show results obtained from the SimaPro model. The table show respective contribution to Ecosystem damage for each production process in the Fish scenario.

Table 17: Ecosystem damage for the Fish scenario, expressed in species.yr / ton oil.

Fish oil	Fishing	Reduction fishery	Transport	Total
Global warming, Terrestrial ecosystems	3,8E-06	1,2E-04	6,9E-06	1,3E-04
Global warming, Freshwater ecosystems	1,1E-10	3,2E-09	1,9E-10	3,5E-09
Ozone formation, Terrestrial ecosystems	3,9E-06	3,3E-05	1,5E-06	3,8E-05
Terrestrial acidification	6,5E-06	7,8E-05	1,9E-06	8,6E-05
Freshwater eutrophication	1,7E-08	6,9E-06	1,8E-07	7,1E-06
Marine eutrophication	5,4E-12	1,2E-09	3,5E-11	1,2E-09
Terrestrial ecotoxicity	9,3E-09	4,3E-07	2,3E-07	6,7E-07
Freshwater ecotoxicity	1,3E-09	3,5E-07	3,9E-08	3,9E-07
Marine ecotoxicity	1,4E-08	1,7E-07	9,2E-09	1,9E-07
Land use	2,2E-08	1,1E-05	5,8E-07	1,1E-05
Water consumption, Terrestrial ecosystem	2,8E-08	4,0E-06	8,2E-08	4,1E-06
Water consumption, Aquatic ecosystems	1,3E-12	2,9E-10	4,3E-12	3,0E-10
Ecosystem damage (Fish oil)	1,4E-05	2,5E-04	1,1E-05	2,8E-04

Biogas	Pre-treatment	AD	Biogas production	Avoided energy	Total
Global warming, Terrestrial ecosystems	1,2E-05	2,4E-06	1,1E-05	-1,1E-04	-8,6E-05
Global warming, Freshwater ecosystems	3,2E-10	6,6E-11	3,0E-10	-3,0E-09	-2,3E-09
Ozone formation, Terrestrial ecosystems	3,7E-06	9,0E-08	7,4E-07	-5,5E-06	-9,4E-07
Terrestrial acidification	3,5E-06	9,9E-06	7,7E-05	-3,9E-05	5,2E-05
Freshwater eutrophication	4,3E-07	4,9E-07	2,8E-07	-3,8E-05	-3,7E-05
Marine eutrophication	7,4E-11	8,0E-11	5,1E-11	-6,1E-09	-5,9E-09
Terrestrial ecotoxicity	1,8E-07	4,8E-09	9,6E-08	-2,1E-07	6,8E-08
Freshwater ecotoxicity	7,4E-08	1,6E-08	2,9E-08	-1,2E-06	-1,1E-06
Marine ecotoxicity	1,7E-08	3,3E-09	6,3E-09	-2,4E-07	-2,2E-07
Land use	3,0E-06	1,6E-07	6,8E-07	-7,7E-06	-3,9E-06
Water consumption, Terrestrial ecosystem	1,2E-07	7,4E-07	4,2E-08	-1,8E-06	-8,6E-07
Water consumption, Aquatic ecosystems	6,2E-12	3,3E-11	3,0E-12	-7,2E-11	-3,0E-11
Ecosystem damage (Biogas)	2,3E-05	1,4E-05	9,0E-05	-2,0E-04	-7,7E-05

In Figure 28 the total Ecosystem damage for the Algae and Fish scenario is illustrated for each contributing midpoint impact.

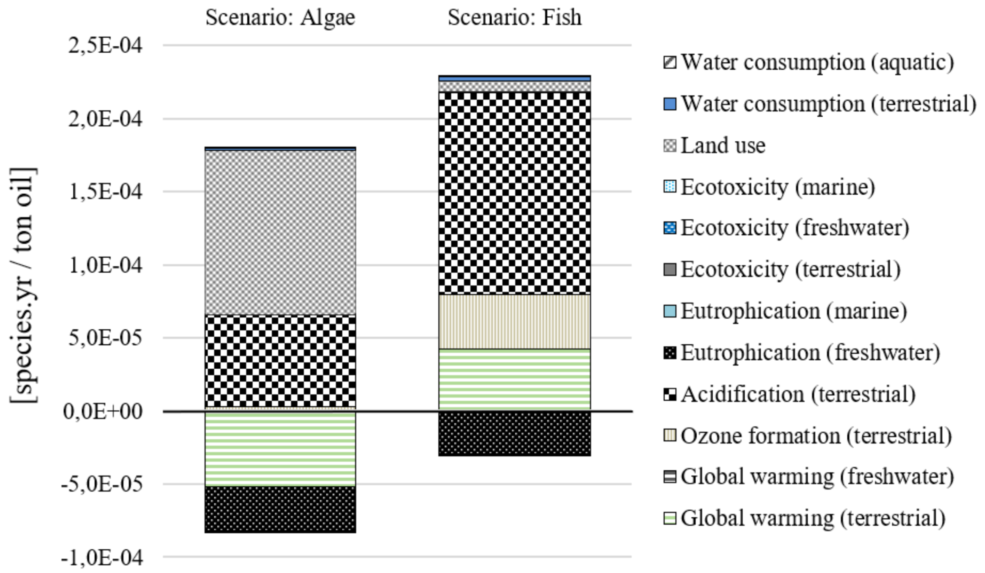

Figure 28: Total Ecosystem damage for the Algae and Fish scenario.

F.3 Sensitivity analysis

This section provides result tables for assessed environmental impact expressed per tonDHA.

Table 18: Environmental impact per tonDHA assuming 100% renewable energy.

		Future energy mix		Modelled energy mix	
	Unit	**DHA_{algae}**	**DHA_{fish}**	**DHA_{algae}**	**DHA_{fish}**
$ADP_{fossil\ fuels}$	TJ / tonDHA	1,2E+00	4,2E+00	-1,9E+00	1,3E+00
GWP_{100}	$tonCO_2e$ / tonDHA	1,2E+02	4,1E+02	-1,9E+02	1,2E+02
Ecosystem damage	species.yr / tonDHA	2,1E-03	2,7E-03	9,7E-04	1,7E-03

Table 19: Environmental impact per tonDHA with optimized VFA production.

		Decreased food waste		Modelled food waste	
	Unit	**DHA_{algae}**	**DHA_{fish}**	**DHA_{algae}**	**DHA_{fish}**
$ADP_{fossil\ fuels}$	TJ / tonDHA	-8,8E-01	3,0E+00	-1,9E+00	1,3E+00
GWP_{100}	$tonCO_2e$ / tonDHA	-8,4E+01	2,9E+02	-1,9E+02	1,2E+02
Ecosystem damage	species.yr / tonDHA	1,2E-03	2,1E-03	9,7E-04	1,7E-03

Table 20: Environmental impact per tonDHA with 10% increased transport.

		+ 10 % transport		Modelled transport	
	Unit	**DHA_{algae}**	**DHA_{fish}**	**DHA_{algae}**	**DHA_{fish}**
$ADP_{fossil\ fuels}$	TJ / tonDHA	-1,8E+00	1,4E+00	-1,9E+00	1,3E+00
GWP_{100}	$tonCO_2e$ / tonDHA	-1,8E+02	1,3E+02	-1,9E+02	1,2E+02
Ecosystem damage	species.yr / tonDHA	9,9E-04	1,7E-03	9,7E-04	1,7E-03