Science is not formal logic it needs the free play of the mind in as great a degree as any other creative art It is true that this is a gift which can hardly be taught but its growth can be encouraged in those who already posses it Max Born (1882-1970)

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Methodological development and case studies

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Summary/Abstract

A growing demand for resources and competition for land use, especially critical for Europe, is fueling the search for renewable and sustainable products. Innovative, resource-efficient and integrated approaches such as the development of marine biomass fed into integrated biorefineries may bring sustainable and cost-effective solutions to meet the growing needs. Algal biomass is no doubt considered a promising and valuable feedstock for a bio-based economy. Algae are fast-growing aquatic organisms and most of them are autotrophs: they convert sunlight, carbon dioxide, nitrogen and phosphorous (often available in excess) into valuable biomass components, which are of interest for a diverse group of industries, e.g., the food, feed, cosmetics and energy sectors (chapter 1). Despite the large potential of products derived from algae, its cultivation in Europe is still in its early stages and an estimation of the environmental sustainability may guide a further development and scale-up of this sector by highlighting its competitiveness (chapter 2). Chapter 3 illustrates the objectives of this PhD dissertation (addressed in Part I).

In Part II, the environmental sustainability (specifically the life cycle resource footprint) of algae production under temperate climate conditions is assessed in an attempt to unravel the bottlenecks of current European production pathways. In chapter 4 and 5, the environmental footprint of microalgae production for higher value applications, and more specific as a feed ingredient, is examined. A first case study concerns an LCA study of microalgae production in Belgium in an innovative cultivation system for aquaculture purposes (chapter 4). Thereafter, the environmental resource footprint of an integrated algal biorefinery located in the Netherlands is assessed (chapter 5). Both case studies consider waste stream mitigation and a comparison was made with the footprint of alternative biomass plants such as soybeans. However, most envFpironmental sustainability assessment methods are rather semi-mature than well-established, which might result in incomplete LCA comparisons. Therefore, Part III of this PhD addresses the need to better quantify the environmental impacts related to surface use, both terrestrial land and sea surface, as this is not straightforward yet in life cycle assessment (LCA). Advanced LCA indicators are proposed in an attempt to better account for the impact of anthropogenic land and sea surface occupation. This development in the field of LCA enables a more fair comparison between the environmental resource footprint of aquatic algae, cultivated on marginal land or in the sea, versus terrestrial crops, of which most of them are grown on fertile land.

Samenvatting/Abstract

De rijzende vraag naar grondstoffen en de concurrentie voor landgebruik, wat vooral een kritiek punt in Europa is, stimuleert de zoektocht naar hernieuwbare en duurzame producten. Innovatie, het efficiënt gebruik van grondstoffen en een geïntegreerde aanpak zoals de ontwikkeling van geïntegreerde bioraffinaderijen met mariene algen kunnen duurzame en kosteneffectieve oplossingen bieden voor de groeiende behoeftes. Algenbiomassa kan zonder twijfel beschouwd worden als een veelbelovende en waardevolle grondstof voor een biogebaseerde economie. Algen zijn snelgroeiende aquatische organismen en de meeste van hen zijn autotrofen: ze zetten zonlicht, koolstofdioxide, stikstof en fosfor (vaak beschikbaar in overmaat) om in waardevolle biomassa componenten die van belang zijn voor een diverse groep van industrieën, bv. de voedings-, dierenvoeding-, cosmetische en energiesectoren (hoofdstuk 1). Ondanks het grote potentieel van de algen-gebaseerde producten bevindt de teelt in Europa zich nog in een vroeg stadium en een inschatting van de milieuduurzaamheid kan de verdere ontwikkeling en opschaling van deze sector stimuleren door de concurrentiepositie te belichten (hoofdstuk 2). Hoofdstuk 3 illustreert de doelstellingen van dit doctoraat (behandeld in deel 1).

In deel II wordt de ecologische duurzaamheid bepaald (in het bijzonder de levenscyclus grondstofvoetafdruk) van algenproductie onder een gematigd klimaat in een poging de knelpunten van de huidige Europese productieketens te identificeren. In hoofdstuk 4 en 5 wordt de milieuvoetafdruk onderzocht van microalgenproductie voor een meer hoogwaardige toepassing, specifiek als voederingrediënt. Een eerste casus betreft een LCA studie van microalgenproductie in België in een innovatief kweeksysteem voor aquacultuur toepassingen (hoofdstuk 4). Vervolgens wordt de grondstofvoetafdruk beoordeeld van een geïntegreerde algenbioraffinaderij gelokaliseerd in Nederland (hoofdstuk 5). Beide studies behelzen het gebruik van afvalstromen en er werd telkens een vergelijking gemaakt met de voetafdruk van alternatieve biomassa zoals sojabonen. Aangezien de meeste beoordelingsmethoden van milieuduurzaamheid nog niet matuur genoeg zijn, kan dit resulteren in onvolledige LCA vergelijkingen. Vandaar dat deel III van dit doctoraat verder ingaat op de noodzaak om milieueffecten in verband met het gebruik van een bepaalde oppervlakte, zowel terrestrisch land als zeeoppervlak, beter te evalueren want dit is echter niet vanzelfsprekend in levenscyclusanalyse (LCA). Daarom worden geavanceerde LCA indicatoren voorgesteld in een poging de milieu-impact van het bezetten van land en zeeoppervlakte door de mens beter te kwantificeren (hoofdstuk 6 en 7). Deze ontwikkeling in het LCA gebied laat een meer eerlijke vergelijking toe tussen de ecologische voetafdruk van aquatische algen, gekweekt op marginaal land of in de zee, tegenover terrestrische gewassen, waarvan de meeste geteeld zijn op vruchtbare bodem.

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List of Abbreviations

ACRRES	Application Centre of Renewable RESources
ALCA	Attributional Life Cycle Assessment
AOP	Area Of Protection
BDP	Biodiversity Damage Potential
BOD	Biochemical Oxygen Demand
BPP	Biotic Production Potential
CED	Cumulative Energy Demand
CEENE	Cumulative Exergy Extraction from the Natural Environment
CEVA	Centre d'Etude et de Valorisation des Algues
CExD	Cumulative Exergy Demand
CF	Characterization Factor
CHP	Combined Heat and Power
CIDI	Compression-Ignition Direct-Injection
CLCA	Consequential Life Cycle Assessment
COD	Chemical Oxygen Demand
CSP	Carbon Sequestration Potential
CW	Center West
DM	Dry matter
DW	Dry weight
EA	Exergy Analysis
EDP	Ecosystem Damage Potential
EF	Ecosystem Function
EFSA	European Food Safety Authority
ELCA	Environmental Life Cycle Assessment
ELCD	European Reference Life Cycle Database
Eq.	Equation
ERP	Erosion Regulation Potential

ESA	Environmental Systems Analysis
EU	European Union
FAME	Fatty Acid Methyl Esters
FAO	Food and Agriculture Organization of the United Nations
FU	Functional Unit
FW	Fresh Weight
FWRP	Fresh Water Regulation Potential
GHG	Greenhouse Gas
GREET	The Greenhouse gases, Regulated Emissions and Energy use in Transportation
	model
GVS	Gram of Volatile Solids
HANPP	Human Appropriation of Net Primary Production
HDPE	High Density Polyethylene
INRA	French National Institute for Agricultural Research
IPCC	Intergovernmental Panel on Climate Change
IEA	International Energy Agency
ILCD	International Reference Life Cycle Data System
IRENA	International Renewable Energy Agency
IS	Improvement Scenario
ISO	International Organisation for Standardization
LANCA	LANd use indicator value CAlculation
LCA	Life Cycle Assessment
LCC	Life Cycle Costing
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LF	Land Function
LUF	Land Use Function
MEA	Millennium Ecosystem Assessment
MEOW	Marine Ecoregions of the World
NASA	National Aeronautics and Space Administration
NDP	Naturalness Degradation Potential
NER	Net Energy Ratio
NOx	Nitrogen Oxides
NPP	Net Primary Production
NPP ₀	The average natural potential NPP
NUIG	National University of Ireland, Galway

NWE	North West Europe
PAR	Photosynthetic Active Radiation
PBR	Photobioreactor
PDF	Potentially Disappeared Fraction
PEF	Pulsed Electric Field
РМ	Particulate Matter
PNV	Potential Natural Vegetation
ProviAPT	Proviron Advanced Photobioreactor Technology
PUFA	Polyunsaturated Fatty Acid
Q	Ecosystem Quality
RAM	Resource Accounting Method
RER	Europe (ecoinvent representation)
SAR	Species-Area Relationship
SED	Solar Energy Demand
SI	Supporting Information
SLCA	Social Life Cycle Assessment
SO	Southern
SOC	Soil Organic Carbon
SOx	Sulphur Oxides
SRS	Solution Recovery Services Inc.
SST	Sea Surface Temperature
UNEP	United Nations Environmental Programme
UN	United Nations
USA	United States of America
USCB	United States Census Bureau
UV	Ultraviolet
VGPM	Vertically Generalized Productivity Model
VOC	Volatile Organic Compounds
WPP	Water Purification Potential
WUR	Wageningen University and Research Centre
Yr	Year

List of Symbols

А	a constant in equation 14
В	a constant in equation 14
BF _{ex}	the overall exergy breeding factor (-)
[chlor.]	the phytoplankton based chlorophyll a concentration (mg chlorophyll.m $^{-3}$)
$CF_{occ,i,j}$	the CF of land occupation impact on ecosystem health for land use type i in country \boldsymbol{j}
	(further referred to zone x)
C _p	heat capacity (kJ kg ⁻¹ K ⁻¹)
C _{tot}	the total pigment content within the euphotic layer
Ex_{ch}	chemical exergy (kJ)
Ex_e	electrical exergy (kJ)
Ex_{eff}	exergy efficiency
Exin	the exergy content of the resources used
Exout	the exergy contained in the final products
$\mathrm{Ex}_{\mathrm{ph}}$	physical exergy (kJ)
Ex _r	radiation exergy (kJ)
Ex_{tot}	total exergy of a product (kJ)
f(par)	the photosynthetic active radiation (PAR) dependent function (/)
HANPP _x	the average NPP impact due to human land use in zone x (MJ $_{ex}m^{\text{-2}}\text{year}^{\text{-1}})$
K _s	the half saturation coefficient (mg dm ⁻³)
М	mass (kg)
NDP_i	the naturalness degradation potential of land use type i (-)
n _i	the naturalness of land use type i (-)
$\text{NPP}_{\text{act},x}$	the average NPP of the actual vegetation in zone x (MJ $_{\rm ex}$ m $^{-2}$ year $^{-1}$)
$\text{NPP}_{h,x}$	the amount of NPP harvested in in zone x (MJ $_{ex}$ m ⁻² year ⁻¹)
$NPP_{0,i}$	the average potential NPP (MJ $_{\rm ex}{\rm m}^{\text{-2}}$ year $^{\text{-1}}$) for grid cell (10 km²) i
$NPP_{0,j}$	the average potential NPP (MJ $_{\rm ex}$ m $^{-2}$ year $^{-1}$) for country j

$NPP_{0,ref}$	the maximum average potential NPP an ecosystem provides worldwide(reference)
$\text{NPP}_{\text{loss},x}$	the NPP loss due to land use in zone x ($MJ_{ex} m^{-2} year^{-1}$)
$\text{NPP}_{t,x}$	the average remaining NPP for the natural environment after land use in zone x
	$(MJ_{ex} m^{-2} year^{-1})$
NPP _{0,x}	the average potential NPP (MJ _{ex} m ⁻² year ⁻¹)
Р	pressure of the substance (K)
P ₀	reference pressure (atm)
Pb _{opt}	the maximum carbon fixation rate within a given water column (mg C mg chlorophyll $^{\mbox{\tiny -1}}$ $h^{\mbox{\tiny -1}})$
S	the concentration of the limiting nutrient (mg dm ⁻³)
Т	temperature of the substance (K)
T _o	reference temperature (K)
R	the gas constant (8.31 J mol ⁻¹ K ⁻¹)
Х	the day of the year with X=1 is December $15^{\rm th}$
X ₀	a constant in equation 14
Y	the radius of the cylinder (m)
z_eu	the euphotic depth equation based on surface chlorophyll concentrations (m)

Greek symbols

$\boldsymbol{\chi}_i$	the mole fractions of the various components in the mixture (-)
γ_{i}	the activity coefficient (-)
Ψ_{a}	the global exergy efficiency (%)
Ψ_{b}	the global exergy efficiency (without solar energy input) (%)
Ω_{a}	the rational efficiency (%)
$\Omega_{ m b}$	the rational efficiency (without solar energy input) (%)
μ	the photosynthetic exergy yield (%)
Н	the algal growth rate (h^{-1})
η_{max}	the maximum algal growth rate (h ⁻¹)
$\Delta \text{NPP}_{\text{LC},x}$	the impact on NPP due to human-induced land conversions in zone x (MJ $_{\rm ex}$ m $^{-2}$ year $^{-1}$

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PART I General introduction

Chapter 1 Algae: the promising biomass?¹

¹ Partly redrafted from

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1.1 Blooming interest in algae

In the late 18th century, a first industrial revolution took place. Machines were made to facilitate daily life and to improve the efficiency of manufacturing in terms of speed and volume. Due to the industrialization, health and welfare of humans increased and the population grew worldwide. However, even after a few decades, people experienced disadvantages and restrictions of this technological development (Meadows et al., 2004). It is proven today that the rapid exploitation of the earth by an increasing number of people has its consequence; resource (e.g., fossil) depletion with price increase as a result, industrial pollution, climate change, increasing land occupation/competition, a larger gap between rich and poor and food and fresh water scarcity in developing countries (Najam et al., 2007; Hanjra and Qureshi, 2010). To deal with these ongoing problems, the day-to-day human lifestyle should be more environmentally friendly and a systematic approach is necessary to sustain our ecosystems. Therefore, the principle of sustainable development on a finite planet has gained wide attention over the last years. The focus has changed from end-of-pipe pollution treatment to resource conservation and clean technology concepts. From this perspective, renewable materials and energy sources are desired (United Nations Environmental Programme (UNEP), 2011). Therefore, the interest in algae was triggered by the fact that it is identified as a renewable resource that can be grown on non-fertile land or in natural water systems. It is a feedstock that can be used for diverse applications with the intention to lower the environmental footprint of the currently used (non-renewable) alternatives.

1.2 Characteristics and background of algae

1.2.1 Habitats and biology

Algae live in an aquatic environment and are typically eukaryotic photo-autotrophs, able to capture light and carbon dioxide (CO_2) which are converted into chemical energy and oxygen. Thanks to their photosynthetic ability, algae have played a major role in the

evolution of the atmosphere; nearly all of the oxygen present today in the atmosphere is produced by algae (Kasting and Siefert, 2002). On top of this, algae form the majority of primary production and provide the basis of oceanic food webs. Thus, they are considered to be one of the most important organisms on Earth. Apart from sunlight and CO_2 , algae are highly dependent on several inorganic macronutrients such as nitrogen and phosphorous that are often found in excess. Also trace elements (e.g., calcium, magnesium) are required for their growth and enzyme activity (McKinney, 2004). Because of these properties, algae are capable of both sequestering CO_2 and purifying nutrient-rich waste streams (Clarens et al., 2010 and Pittman et al., 2011).

Diverse types of algae exist, ranging from single-celled microalgae floating in the water to multicellular, large macroalgae or seaweeds (both attached and free-floating forms are common). Moreover cyanobacteria exist, also called blue-green microalgae. However, cyanobacteria are prokaryotic and their relatively simple, primitive life form is closely related to bacteria. Therefore, many authors do not consider cyanobacteria as being part of the algae domain (Pandey et al., 2014). While most species live in marine conditions, a few algal species thrive well in brackish water or freshwater (Hurd et al., 2014). Most species of macroalgae can be found in coastal regions where they attach to fixed substrates (bedrock, boulders etc.) under suitable light and nutrient (upwelling) conditions (Taelman et al., 2014). In the oceans, nutrient limitation is likely to occur at the surface due to upwelling of nutrient-rich water from the deep ocean, whilst light limitation occurs in the water body under the euphotic zone which is the upper surface layer (± 200 m). The coastal regions and lakes or ponds have usually both resources (light and nutrient) available in adequate amounts: these shallow waters allow light to penetrate to the bottom and nutrients are provided through run-off from land (Taelman et al., 2014).

Depending on the algal species, sexual or asexual reproduction takes place (Figure 1). Concerning asexual reproduction, the most common techniques used by algae are: multiple fission where the parent cell can be divided in more identical daughter cells, sporulation (spore formation and release) and fragmentation (a new organism grows from a fragment of the parent). In sexual reproduction, genetic material from two individuals is combined. Conjugation is a possibility to exchange genetic material during the fusion of two similar organisms. Another form of sexual reproduction involves zygote forming, which is used by most macroalgae (Ibrahim, 2007).



Figure 1 Schematic presentation of the most common reproduction pathways for algae. Asexual: multiple fission, sporulation and fragmentation. Sexual: conjugation and zygote forming.

In general, microalgae are a very rich source of biomass, containing large amounts of proteins (6-52% DM), carbohydrates (5-23% DM) and lipids (7-23% DM) with essential ω -3 and ω -6 polyunsaturated fatty acids (PUFA), minerals, carotenoids and vitamins (Becker, 2004; Borowitzka and Moheimani, 2013). In contrast, the approximate proportions of different types of seaweed are ca. 7-35% DM proteins, 45-75% DM carbohydrates and fibers, less than 5% DM fats and several minerals and vitamins, resulting in high ash contents of 9-44% DM (Mouritsen, 2013). Depending on the species, they also contain vitamins (e.g., provitamin A, vitamin B12, tocopherols), carotenoids (e.g., asthaxanthin, β -carotene,
canthaxanthin, lutein), minerals (e.g., calcium, iodine), nutritional proteins and typical carbohydrates (e.g., alginates, mannitol, xylan, fucoidan, laminaran, agar, carrageenan and mannan). However, on average, the moisture content of fresh seaweed is very high and may amount to 94% of the biomass (Van den Burg et al., 2013). According to their nutritional value, chemical composition and pigmentation, they are classified in three diverse phyla: Ochrophyta (brown seaweeds), Rhodophyta (red seaweeds) and Chlorophyta (green seaweeds) (Borowitzka and Moheimani, 2013; El-Said and El Sikaily, 2013; Mouritsen, 2013). Overall, the chemical composition of algae vary considerably with the species used and seasonality as well as geographical location.

1.2.2 Aquatic algae versus terrestrial plants

The photosynthetic mechanisms of algae are similar to that of terrestrial land-based plants but, generally, they are more efficient in converting sunlight into biomass because of a less complex cellular structure and their direct access to water, nutrients and CO_2 (Kilinç et al., 2013). According to Li et al. (2008), algae have the ability to capture solar energy with an efficiency of 10-50 times higher than that of most land-based plants, which is correlated to a higher bio-fixation of carbon dioxide (the most common greenhouse gas, (GHG)). About 1.83 kg of CO_2 can be fixed to produce 1 kg of algal dry cell weight (Chisti, 2007; Pandey et al., 2014). Additionally, algae can take up water and nutrients more efficiently which leads to a significantly (factor 10 to 1000) faster growth than terrestrial plants (Wang, et al., 2008). A study of Griffiths and Harrison (2009) revealed that green microalgae have an average doubling time of about 24 h and the exponential growth phase can be as short as 3.5 h. Other taxa, such as cyanobacteria, have even lower average doubling times of 17 hours. Seaweeds have, in general, higher doubling times of a few days (Jackson, 1980). Though, the algal productivity (kg m⁻² year⁻¹) could be lower than land-based plants because of the low algal cell densities (Kirchman, 2012).

Because of scarce land availability these days, algae production can be a good alternative compared to terrestrial plants because it is possible to cultivate the microalgae in artificial ponds or photobioreactors (PBRs) on nutrient-poor, marginal land, unable to support traditional agriculture (Posten and Walter, 2012). Thus, competition with food crop

cultivation can be avoided. Seaweed can be cultivated offshore, near the coast, where sufficient amounts of nutrients are available. Shifting production to the sea can contribute to address one of the most important challenges, which is land use competition, as this has risen because of the growing human population (Van den Burg et al., 2013).

Compared to terrestrial plants, the biochemical composition of microalgae and seaweeds is significantly different. For example, most land-based cell walls contain lignin, a complex molecule that contributes to plants' structural strength. The absence of lignin in most algae (except for some red algae) makes them an attractive feedstock for bioethanol production. In addition, both micro- and macroalgae are a very rich sort of biomass with a high nutritional value as they contain, amongst other components, essential omega-3 and omega-6 polyunsaturated fatty acids (PUFA), vitamins, carotenoids, minerals, nutritional proteins and carbohydrates (Brown et al., 1997; Mouritsen, 2013; Borowitzka and Moheimani, 2013). From this perspective, it is clear that algae have a lot of potential compared to terrestrial plants that lack (or have only small amounts of) valuable components such as vitamin B12 and ω -3 and ω -6 fatty acids (Croft, 2005; Ward and Singh, 2005).

1.2.3 Algal cultivation and processing methods

The potential of algae leads to the demand for controlled cultivation and processing methods. This has encouraged the search for specific systems in which algae can be produced with the highest possible efficiency.

1.2.3.1 Cultivation systems

Algae cultivation systems differ quite substantially depending on the type of algae (microalgae and macroalgae) which is being produced.

<u>Microalgae</u>

Two main microalgae cultivation systems exist: open (raceway) ponds and closed PBRs, both having their own advantages and limitations (Table 1). The choice of often depends on the type of algae (requirements for specific environments) and the final application

that is pursued. The most common open systems for microalgae production are tanks or shallow ponds, often in the form of a raceway and usually with a depth of 0.2-0.5 m to ensure that algae receive adequate exposure to sunlight (Campbell et al., 2011). The ponds can be built in concrete or simply constructed with plastic liners (Collet et al., 2015). Often a paddle wheel is used for circulation and mixing (Rogers et al., 2013). Open ponds are claimed to be rather inexpensive, have a simple technology and generally consume less energy compared with the closed systems. These systems are the most industrially applied (several acres) in countries such as Brazil, Hawaii, India and USA and are mainly being used to cultivate Spirulina (a filamentous cyanobacterium with a high protein content), Chlorella (high protein content), Dunaliella (high β -carotene content) and Haematococcus (high astaxanthin content) (Pandey et al., 2014). However, an important disadvantage is the lower biomass productivity due to a lower controllability of evaporation losses, light intensity, temperature, pH, and dissolved oxygen concentration, which leads to higher harvesting and downstream processing requirements. Especially in temperate climates, where suitable sunlight intensity is not always available throughout the year, the productivity may be very low (Pandey et al., 2014). In addition, contamination by other microalgae and microorganisms may occur. Therefore it is important to cultivate particular algae able to survive under extreme environmental conditions to ensure the existence of monoculture in a pond. *Chlorella*, for example, can grow well in a nutrient-rich medium, Spirulina grows favorably at high pH and bicarbonate concentration, and D. salina is well adapted to a highly saline medium (Brennan and Owende, 2010).

In an attempt to overcome the main limitations of open ponds, several types of PBRs are developed. However, they struggle with higher energy use and costs (Jorquera et al., 2010). Though, for higher value applications that require a high controllability of the biomass quality, PBRs are stated to be a better choice (Pandey et al., 2014). Examples of these reactors include flat plate PBR, tubular PBR, column PBR, large plastic bags (e.g., the helioreactors of Campanella et al., 2012) and membrane PBR (Wang et al., 2012). They can be operated in both batch and continuous modes. Despite the wide variety of PBR configurations developed, there is still a need for further optimization. Lower energy requirements and higher biomass yields should be achieved in a system that is easy to scale-up (Jorquera et al., 2010).

During cultivation, manipulation of the biochemical composition of the microalgae by changing the environmental growth conditions (pH, temperature, supply of CO_2 and nutrients, illumination, etc) can be performed, but the effects can vary between species (Brown et al., 1997). In general, stress conditions (especially nitrogen-deficiency) lead to lipid accumulation but as a constraint, it affects the growth rate negatively. When aiming at high lipid productivities, it seems better to cultivate the algae in a two-step process: a nutrient sufficient phase to produce enough cells (e.g., in open ponds), followed by a nitrogen insufficient phase to enhance lipid synthesis (preferably in a controlled PBR) (Rodolfi et al., 2008).

To conclude, an ideal microalgae cultivation system should meet the following requirements: (1) a large effective illumination area, (2) simple to operate, (3) low contamination risks, (4) optimal gas-liquid transfer rates, (5) low capital and operating costs, (6) easy to scale up and (7) a high areal productivity, i.e., uses a minimal amount of land (Xu et al., 2009). Unfortunately, even more intensive research and field trials are necessary to realize this ideal cultivation system.

	Benefits	Limitations
Open ponds	Relatively inexpensive	Lower biomass conc. $(0.1-0.5 \text{ g L}^{-1})$
	Easier to clean	Processing is difficult due to low algae
	Uses marginal land	concentrations
	Lower energy input	More land area necessary
	Easier maintenance	Limited to some algae species
	Oxygen concentration low enough	Poor mixing, light and CO_2 use
		High contamination risk
		Water loss due to evaporation
		Weather dependent (rain)
		Difficult upscaling
Closed	Higher biomass conc. (1-8 g L ⁻¹)	Higher energy consumption
photobioreactors	Lower contamination risk	Relatively expensive
	Less space required + marginal land	Problems with fouling
	Losses limited (water, CO ₂ ,)	Need for gas exchange (toxic O_2)
	Better biomass control and quality	Need for cleaning to remove wall growth
	Simpler scale-up possible (e.g., bags)	Need for cooling in warm climate
	Less weather dependent	conditions
	Easier processing due to higher	
	concentrations	

Table 1Benefits and limitations of open and closed microalgae cultivation systems (Chisti,
2007; Brennan and Owende, 2010; Pulz, 2001).

Macroalgae

Worldwide, almost 21 million tonnes of seaweed is utilized, of which 94% is produced in aquaculture, the rest being harvested from the wild (Tiwari and Troy, 2015). To reproduce some types of seaweeds, especially many brown seaweeds (e.g., the Laminariales), controlling the sexual life cycle of the seaweed is the only way (McHugh, 2003). Often, the plantlet production is located onshore, in a hatchery where tanks or ponds are used to produce seedlings in a controlled environment, which is far more energy-intensive than the grow-out phase at sea. Monitoring the reproductive cycle of gametophyte and sporophyte phases is essential for a successful cultivation; it requires greater control of the life cycle than seaweeds that are grown vegetatively. The latter can be grown by taking cuttings from mature ones, which may take place near-shore in a natural environment.

The majority of seaweed biomass available on the market is cultivated at sea (offshore or nearshore) in their natural environment. Typically, the production is dominated by cultivation techniques that produce large amounts of monospecific biomass (Hasting et al., 2015). Worldwide, there are at least four seaweed cultivation methods demonstrated; the bottom planting method, the line method, net cultivation, and the raft system (Andersen, 2005; Tiwari and Troy, 2015). All cultivation techniques are relatively labor-intensive.

The bottom planting method involves the cultivation of seaweeds on substratum placed directly on the sea bottom. This method is typically employed in areas where low level of water remains at low tides such that the planting can be performed without diving and is only suitable for some benthic genera (Felix, 2013). For the line method, seaweeds are attached to ropes (usually 10 to 50 m long) that are placed in parallel arrangement with varying spaces between them. Three types of longline cultivation exist. The most used one is the off-bottom method because of its simplicity, cheapness, easy installation and maintenance. Stakes, usually made of wood, are used to hold the ropes that are approximately 10 meters long (Sahoo and Yarish, 2005; Valderrama et al., 2013). This method therefore requires an appropriate shallow site (e.g., lagoons) with sandy bottom and sufficient sunlight. At low tides, the ropes get exposed. At that moment, the farm is easily accessible by foot. One potential problem is the threat of intertidal epiphytes and grazers, i.e., sites have to be carefully chosen for a successful implementation (Foscarini and Prakash, 1990). The second option is to submerge the line at midwater level near the shore. In contrast, floating longline methods are used in deeper waters, further from the shore. This indicates the need for a boat for access, the anchoring of lines to the sea bottom as well as the use of buoys to maintain stability in the water column (Tiwari and Troy, 2015). Net cultivation is analogous to line cultivation, at different depths and locations with the only difference that nets are used instead of single lines. Additionally, a raft system exists, constructed rom floating material (e.g., bamboo or plastic) that serves as a basis for the attachment of the seaweed culture ropes or nets.

All these ocean-based operations need to deal with the major risk of epiphyte overgrowth and are subjected to continuous changes in weather and ocean conditions, which may affect the biomass quantity and/or quality (Hafting et al., 2012). For example, bioactive compounds can fluctuate seasonally, geographically and bathymetrically (Hafting et al., 2015). Therefore, to avoid unstable cultivation conditions when aiming for high-value seaweed products, on-land production units will become more common so that the consistency and quality of the seaweed biomass can be more closely controlled. However, on-land cultivation tanks have specific challenges such as high costs and the availability of suitable coastal land (Hafting et al., 2012).

1.2.3.2 Harvesting and processing of algal biomass

Because microalgae have a small cell size and are only available at low concentrations (0.1 - $8\ g\ L^{\mbox{--}1}$ dependent on the type of reactor, environment, species, etc.) compared to terrestrial plants, intensive harvesting and processing methods are required to separate the microalgal biomass from the culture medium and to concentrate the biomass below a desired moisture content (Pandey et al., 2014; Pulz, 2001). The most common harvesting methods are filtration, flocculation, flotation, sedimentation and centrifugation (Wang et al., 2015). The selection of an appropriate harvesting method depends on the properties of the microalgae, the cell density, size and the desired specifications of the final product. Most of these methods are very energy-intensive and/or have high capital costs (Aitken and Antizar-Latislao, 2012), e.g., gravity sedimentation consumes in general the least energy compared to other harvesting methods but for a commercial-scale (>4 hectares) algae cultivation process and considering the slow sedimentation rates of algae, multiple tanks of large volumes may be required. In contrast, centrifugation is a very efficient technique, but the high energy consumption clearly eliminates it as an option for harvesting a low-value energy crop, on both cost and energy grounds. The residual harvesting methods (flocculation/ filtration/ flotation) are mainly less energy-intensive than centrifugation but are in general less efficient harvesting options (Singh et al., 2013).

In order to find a balance between efficient harvesting and low costs, often multiple separation steps are used to concentrate the biomass after harvest, e.g., first microfiltration to retain the biomass followed by centrifugation to thicken the biomass stream (Brennan and Owende, 2010; Weschler et al., 2014). Certainly, energy-efficient and cost-effective harvesting are two major challenges in the commercialization of algae products. Most of the algae-harvesting techniques present several disadvantages, not only

because of the associated high costs of operation but also due to the frequently low separation efficiencies and the poor product quality. Sedimentation, centrifugation, and filtration processes involve the use of equipment that could result in deterioration in algal quality due to cell rupture that causes leakage of cell content. Furthermore, in the case of flocculation, the use of coagulants can have a negative effect on the quality of the final product.

The harvesting procedure of seaweeds is, in contrast to microalgae, more labor-intensive (often not automated) but requires less energy. The techniques to remove the full grown seaweeds from the cultivation systems are simple but time consuming (Mouritsen, 2013). Identifying the best harvest times is dependent on the type of species, the environmental conditions, the production cycle and the season. Analysis of the effect of seasonal variation on the chemical composition of seaweed can be used to determine the optimal harvesting time related to components of most interest commercially. For example, it was reported that the highest alginate concentration in *Saccharina latissima* was found in September, which indicates the importance of harvesting in September for the phycocolloid industry in Europe (Schiener et al., 2014). Nevertheless, for human food, it seems better to harvest at the end of the spring season when the quality of the biomass is still high and not affected by epiphytes (Hurd et al., 2014).

After harvesting the biomass, it is important to quickly process the biomass to avoid degradation. Because the costs of thermal drying (e.g., drum dryer) are higher than mechanical dewatering, it seems essential to dehydrate the biomass as much as possible in the first stages of harvest and processing. Several types of drying are available in order to conserve the algal biomass for a longer period of time; spray drying, drum drying, freeze-drying; belt drying and sun or wind-drying, although it is stated that the latter is not very effective for microalgae (Mata et al., 2009). Furthermore, cell disruption and extraction techniques can be used to release the metabolites of interest (Steriti et al., 2014; Pragya et al., 2013). These techniques strongly depend on the desired products and are briefly discussed in section 1.2.4.

Most technologies for algal growth, harvesting, and conversion are operational at a pilot scale (several squared meters), especially in Europe. Pilot scale plants demonstrate the robustness and scalability of the technology, providing the degree of confidence that is required to secure the investment to take the technology to the next level. However, limited data exists about the feasibility of these technologies being able to operate at a commercial scale (Handler et al., 2014).

1.2.4 The potential applications of algae

Algae are some of the oldest life forms on earth, but they have only recently been recognized as a very promising (but challenging) source of biomass for the biobased economy. Although the total number of algal species is rather uncertain, estimates predict that several million algal species exist. This large diversity in chemical compositions, morphology, living environment and reproduction methods therefore offers a large range of potential applications (Guiry, 2012). Focus can be on the production of algae for food, feed and energy. Also in the chemical industry, specific algal components can be used as cosmetic ingredients, chemical building blocks and pharmaceutics (Sing and Gu, 2010) (Figure 2).



Figure 2 Potential market applications of algae production

Apart from their potential as final product in a wide range of industries, it is also possible to aim at pollution control; making use of algae for wastewater treatment and carbon mitigation. The production of algae (whether or not with the use of waste streams) in a biorefinery where all biomass components are utilized to produce several products seems a very promising pathway towards commercialization.

Pollution treatment

Pollution is a man-made phenomenon, but algae can be used as an end-of-pipe treatment to mitigate this problem. Efficient capture of CO_2 , which is the GHG emitted by human activities, is an important strategy in the reduction of atmospheric CO_2 levels. Compared to photosynthetic terrestrial plants, micro- and macroalgae are important primary producers (mostly autotrophs) that grow much faster and their CO_2 -fixation efficiency seems 10-50 times better, i.e. they assist in regulating the effect of climate change by consuming CO_2 for growth. Their high potential to mitigate CO_2 emissions makes them suitable to treat industrial exhaust gases as targets for reducing GHG emissions are becoming more strict. Furthermore, carbon can be stored for a long time in the sediment due to the burial of dead algae (Raven and Falkowski, 1999).

Obviously, the performance of the algal strains is related to the culture conditions (light intensity, CO_2 concentration, temperature, reactor design, etc.). For example, when the temperature increases, the CO_2 solubility decreases and the fixation rate decelerates (Ho et al., 2011). Improvements of biomass yield can be noticed when flue gases are used as feed but a careful algae strain selection based on tolerance for high temperatures, high CO_2 concentrations and toxic compounds (e.g., nitrogen oxides, NO_x) is necessary to prevent growth inhibition (and reduced CO_2 -mitigation).

Moreover, algae can play an important role in reducing nitrogen and phosphorous compounds that are often present in excess in coastal waters and in several types of wastewater, i.e. they have the potential to reduce eutrophication or purify wastewater (Leston et al., 2008; Morand and Merceron, 2005). The uptake of nutrients from wastewater for algal growth would eliminate the use of fertilizers derived from fossil-fuel energy, thus mitigating emissions. Because of the pollution treatment potential of algae, farmers are looking to tie into existing infrastructures, such as coal-fired power plants or sewage

treatment facilities. To reduce eutrophication in coastal areas, seaweeds may be chosen over microalgae because the latter have a short lifespan and end up as dead cells in the water very fast. The dead biomass is then decomposed by bacteria, whereby the nutrients will be released back into the sea. In addition, a low dissolved oxygen environment will be created which may harm many aquatic organisms. Therefore, seaweeds have more potential to decrease the eutrophication problem because they can more easily be harvested (Fei, 2004). As a result, there is growing interest in combining seaweed production with caged fish aquaculture to improve water quality in coastal areas, something that has been called integrated multitrophic aquaculture (Tiwari and Troy, 2015)

Both micro- and macroalgae assimilate organic pollutants into cellular components such as lipids and sugars and produce oxygen at the same time that can be used by bacteria that perform an additional nutrient removal (Aitken and Antizar-Latislao, 2012). In case water is polluted with heavy metals such as Cd (II), Ni (II), Mn (II) and Cr(IV) ions, it is possible for some selected species of seaweed to immobilize heavy metal ions due to their specific sorption capacities (Hashim and Chu, 2004). In addition, nonliving microalgae are able to bind the ions passively to their cell surface (biosorption).

Algal-based bioenergy

The burning of high amounts of fossil fuels lead to an ongoing need for renewable raw materials. Therefore, research into third generation biofuels, including algal fuels, is intensified globally. Different fuels can be produced from algae: biodiesel (except from seaweeds due to their low amount of extractable lipids), bioethanol, biogas, bio-oil, syngas, and hydrogen. Especially the substitution of conventional motor fuels by biodiesel from microalgae has gained interest in recent years.

<u>Biodiesel</u>

Among the various fuel possibilities derived from microalgae, biodiesel received the most attention because it shares similar chemical characteristics with petrol diesel and can be directly channeled into the current transportation infrastructure without major modifications to the existing technology and fuel pipelines (Pandey et al., 2014). To produce biodiesel, the lipid content and quality is important and, in many cases, organic solvents are used to extract the oil. Benzene, hexane, cyclohexane, acetone, methanol, ethanol and chloroform are effective solvents that degrade the cell membranes and extract the oil. To perform well in industry, these solvents must be cheap, efficient, insoluble in water, reusable and non-hazardous. Methanol-chloroform 1:1 is the preferred mixture for extraction of total lipids (Ryckebosch et al., 2012). Solvent extraction is often associated with mechanical disruption techniques in order to improve oil yields: bead milling, pressing and homogenization for example are commonly used. Typically, bead milling is the most effective and energy efficient cell disruption technique for algae concentrations between 100 and 200 g L^{-1} (Greenwell et al., 2010).

Also supercritical fluid extraction can be used to remove lipids from the residual biomass. Carbon dioxide is a very suitable supercritical fluid because of its low critical temperature (304 K) and pressure (72.9 atm). Compared to organic solvent extraction, this technique is rather expensive, has a high energy consumption and is difficult to scale up. Other promising oil extraction technologies are ultrasonication, which is based on cavitation, enzymatic treatment, pulsed electric field technology (PEF) and microwave technology (Mercer and Armenta, 2011).

After oil-extraction, the vegetable oil must be processed for biodiesel production. Transesterification of wet algae is the most commonly used method because of its low cost and production of high quality diesel compared to techniques such as pyrolysis. A reaction between microalgal lipids and an alcohol (e.g., methanol) in the presence of a catalyst leads to the production of fatty acid methyl esters (FAME) and glycerol as byproduct.

Another possibility, which is proven to be cost-effective and efficient in terms of methyl ester yield, is in situ or direct transesterification of dry algae. This technique combines extraction and transesterification: the extractant and reactant can be the same solvent, e.g., methanol (Pragya et al., 2013). According to Gouveia and Oliveira (2009), the biodiesel produced is much cleaner than petroleum diesel because it is virtually free of sulfur and there are less emissions of hydrocarbons, carbon dioxide and particulates during combustion. Algal biodiesel can be blend with petroleum diesel or can be used in existing diesel engines without any modification.

<u>Bioethanol</u>

Algal biomass (or residuals available after extraction of valuable components such as oil and oxidants) can be further processed into bio-ethanol. A (mechanical or enzymatic) disruption of the cell wall is necessary to allow a conversion of carbohydrates and starches into sugars. Afterwards, the sugars are fermented by yeasts (typically Saccharomycess cerevisiae). A distillation and purification step is required to increase the ethanol concentration to about 98% (v/v) and to produce a fuel applicable to existing engines. As an alternative, intracellular ethanol production is a promising concept where algae are cultivated in a closed, anaerobic environment. Fermentation is an optimized process for many feedstocks but ethanol production from algae is still under-researched (Aitken and Antizar-Latislao, 2012; Pragya et al., 2013; Thi Hong Minh and Van Hanh, 2012).

<u>Biogas</u>

Anaerobic digestion of algal biomass delivers two useful products: bio-energy in the form of biogas, mainly composed of methane and carbon dioxide, and digestate that can be used as a fertilizer. The theoretical methane yield can vary between species; lipids have a higher methane production potential than proteins and carbohydrates. Though, hydrolization of lipids is slower than that of proteins and sugars (Pragya et al., 2013). Therefore, when the lipid content of the microalgal cells is 40% of more, it seems better to extract the lipids before digesting the residual biomass. This combined process of lipid extraction to produce biodiesel and anaerobic digestion of residual biomass may increase the overall energy yield.

A study of Vanegas and Bartlett (Vanegas and Bartlett, 2013) about co-digesting of seaweed indicates that biogas production from seaweeds at high yields is possible; a methane yield of 244 ml per g volatile solids (gvs) is achieved through co-digestion of *Saccharina latissima* with bovine slurry which is higher than grass (168 ml gvs-1) but lower than rice (264 ml gvs⁻¹). Nevertheless, obtaining energy from seaweeds is not yet available on a large commercial scale (Aitken et al., 2014). In general, biogas is suitable as a fuel for many purposes without processing, except for use in engines because of the corrosive hydrogen sulphide and the presence of CO_2 . Scrubbing (washing the gas with pressurized water) is a technique often used to remove these components (Aitken and Antizar-Latislao, 2012).

Other energy applications

Gasification of algae is a hydrothermal process which delivers syngas; a mixture of several compounds such as methane, carbon monoxide, carbon dioxide, hydrogen and nitrogen. This process operates under high steam temperatures typically ranging from 700 °C to 1100 °C (Chen et al., 2009) and under a limited amount of oxygen (no complete combustion). A drying step in advance is not necessary when the moisture content is below 50%, implying the need for drying seaweeds. Syngas can be burned to produce heat or is used in gas engines and turbines to produce electricity (Pragya et al., 2013).

Pyrolysis is the conversion of biomass into char, liquid (bio-oil) and gaseous products through a heating process in the absence of oxygen. Bio-oil is often the most attractive end product as it has a higher energy density and is easily transported and stored. Slow, fast and flash pyrolysis processes exist, classified by temperature and process time. The process can be adjusted to favor charcoal, oil or gas production (Milledge et al., 2014). According to Demirbas (2011), the yield of bio-oil and gaseous products increases with elevated temperatures whereas the amount of biochar decreases. When bio-oil is the primary product, flash pyrolysis (about 500°C and vapor resistance times of 2-3 seconds) is often used. The liquid fuels from fast pyrolysis of algae are of higher quality than that of lignocelluloses and can be used in many applications, e.g., as direct substitutes for conventional fuel (Amin, 2009). Importantly, the lipid content of the algae is believed to influence the energy balance of pyrolysis; it is indicated that a higher lipid content improves the energy balance (Milledge et al., 2014), therefore pyrolysis of microalgae may be more favorable than for macroalgae.

Another technology to convert wet biomass material to liquid fuel (bio-oil) under moderate temperatures (± 300°C) and high pressures (15-20 MPa) in the presence of a catalyst (e.g., alkali salts) is direct hydrothermal liquefaction. Dichloromethane is often used to separate the oil fraction from the liquefaction products. The oil is lower in oxygen and is a more stable product than the oil obtained from pyrolysis. In addition, drying of biomass after harvesting is not required (if moisture content is below 90%) prior to liquefaction. However, the commercial interest in this technique is low due to the higher costs compared to pyrolysis and gasification (Milledge et al., 2014). Although this seems an effective method to produce biofuels from algae, more research is required in this field (Demirbas, 2011).

Another form of energy from specific algae is biohydrogen (H_2) production. The process of photosynthesis is the fundamental driving force; fixation of carbon dioxide leads to the production of carbohydrates, which are rich of hydrogen atoms. A next phase of complete darkness in absence of oxygen (caused by e.g., sulphur deprivation) induces the synthesis/activation of certain enzymes, e.g., [FeFe]-hydrogenases, responsible for H_2 production (indirect photolysis). Some green microalgae such as *Scenedesmus obliquis, Chlamydomonas reinhardtii* and *Chlorella fusca* are able to immediately dissociate water molecules under sunlight and anaerobic conditions to produce hydrogen gas (direct biophotolysis) (Sharma et al., 2013).

Biobased products from algae

Apart from their potential for pollution control and energy production, considerable efforts have been made to introduce algae in other industrial sectors such as the food, feed, pharmaceutical, chemical and cosmetic industries. Today, the consumption of algae (especially seaweeds) as a low-calorie, high nutritional value source of human food is by far the largest commercial application (FAO, 2013; Kilinç et al., 2013). Algae are used in the food industry as a nutritional supplement or a food colorant. Carotenoids such as astaxanthin, β -carotene, lutein, lycopene, zeaxanthin and bixin are mainly used for food coloring.

In addition to their nutritional value, algae may have potential beneficial effects on human health (nutraceuticals); protection against oxidative stress, anticancer activity, antiinflammatory (e.g., for asthma, prevent muscle damage), etc. (Mata et al., 2009). The beneficial action of some edible algae is due to therapeutic properties of some biologically active components such carotenoids, phlorotannins, fucoidins, laminarins and peptides that are not (or at lower concentrations) present in conventional food (Becker, 2004). Algae for human nutrition are marketed in different forms such as tablets, powders, capsules and liquids or incorporated in food products such as candy, ice cream, tea and bread (Spolaore et al., 2006). Requirements of algae for food and feed applications are, amongst others: non-toxicity, digestibility and containing interesting biochemical constituents (Mata et al., 2009). Especially efforts related to biomass safety for food/feed applications were made in the EU after the food-related crisis that appeared during the 1990s; a European Food Safety Authority (EFSA) was set up and several laws were established e.g., regulation (EC) 178/2002 on food safety (general principles and requirements) and regulation (EC) 258/97 on novel foods and ingredients. Also legislation on animal feed was developed and it provided a framework for ensuring that feedstuffs did not present any danger to human or animal health or to the environment. It includes rules on the circulation and use of feed materials, requirements for feed hygiene, rules on undesirable substances in animal feed, legislation on genetically modified food and feed and conditions for the use of additives in animal nutrition (Enzing et al., 2014; Vos, 2000).

When aiming for feed applications, algae are mainly used in aquaculture for the rearing of larvae, zooplankton and juvenile fish (Taelman et al., 2013). In addition, the use of algae in conventional feed for pets and agricultural livestock gets great attention nowadays because of the emerging health and food/feed safety concerns and the search for sustainable protein supplies (Petrick et al., 2013). In the food and feed industry it is common to use full algae cells (fresh or dried), which is often not the case in sectors such as the pharmaceutical industry, chemistry and cosmetics where high value molecules are extracted from the cells. These compounds are pigments, antioxidants, fatty acids, vitamins, polysaccharides and triglycerides, difficult to produce synthetically. Also alginate, agar and carrageenan (phycocolloids) from seaweeds are often used in the food, pharmaceutical, cosmetics and chemical industries as stabilizer, bulking, thickening and gelling agents (Dhargalkar and Pereira, 2005). The production of industrial chemicals, bioplastics and fertilizers from algae is still in its early stages, but breakthroughs are to come (Mata et al., 2009).

Algal biorefineries

A biorefinery is a facility that integrates biomass conversion processes and equipment to produce multiple products such as fuels, power, and chemicals from biomass. According to Khan et al. (2009) and Gnansounou and Raman (2016), an integrated algal biorefinery is an efficient approach to reduce the overall (economic and environmental) costs of algae production because all components of the biomass are used to produce desirable products

(Vanthoor-Koopmans et al., 2013). Algae can be cultivated with the use of effluent waters from agro-industries and/or carbon rich non-toxic flue gases from power generation facilities with the intention to produce fertilizers, animal feed, healthcare products, electricity, etc. (Figure 3). It seems a win-win situation; on the one hand waste streams are diluted and on the other hand, economic valuable products are produced. The successful deployment of algal biorefineries will eventually be determined by both economic viability and environmental sustainability.



Figure 3 Example of an integrated algal biorefinery (Khan et al., 2009)

Chapter 2 The environmental sustainability of algae production

2.1 Environmental life cycle assessment

Although algae are claimed to be a sustainable resource, there has been an increasing awareness of the possible impact of algae production on the natural environment. The most used framework is a comprehensive state-of-the-art life cycle assessment (LCA), a tool used to quantify all relevant emissions and resources consumed, as well as the related environmental impacts and resource depletion associated with a product's life cycle. LCA takes into account the full lifecycle: from raw material extraction through materials processing, manufacture, distribution, use, repair and maintenance, and disposal or recycling (European Commission, 2010b; Rebitzer et al. 2004). Environmental LCA (ELCA) focuses on the interactions between the cradle to gate or the full cradle to grave chain in the technosphere and the natural environment (De Meester, 2013). Hence, LCA makes it possible to identify opportunities to improve the environmental footprint of products at different phases of their life cycle. It can be used for decision makers in industry and (non-) governmental organizations (ISO, 2006b).

The International Organization for Standardization (ISO) provides ISO 14040 and 14044 guidelines for conducting a conventional LCA (Figure 4). Four main phases are defined as follows: 1) goal and scope definition, the phase where the functional unit and product system are determined, 2) the life cycle inventory (LCI) step where the elementary flows (emissions to and resources from nature) that are attributed to a specific product or service are quantified, 3) the life cycle impact assessment (LCIA) step where the LCI resources and emissions are translated into their respective environmental impact at global and/or regional scales and 4) the interpretation of the results where conclusions can be formulated and improvements proposed (ISO, 2006a, 2006b; Lédon et al., 2012; Souza et al., 2015).



Figure 4 LCA as a 4-phase process according to the ISO standards 14040: goal and scope definition, inventory analysis, impact assessment and interpretation

During the first phase, the purpose of the study has to be clearly defined. In addition, the scope should describe the detail and depth of the study, and show that the goal can be met with the actual extent of the limitations. The following aspects should be considered and described: the product system, the functions of the product system, the functional unit and reference flow, the system boundaries, possible allocation procedures, the environmental impact assessment methodology, the data (quality) requirements and the assumptions and limitations. Through the iterative character of the LCA, adjustments can easily be made during the LCA analysis.

The goal and scope definition is critical in accurately drawing a boundary for an LCA. The system boundaries describe which processes within the life cycle of a product are included, to provide the function of the system, defined by the functional unit. The functional unit provides a reference to which all material and energy inputs/outputs and waste outputs (quantified during the data inventory step) are normalized (Hou et al. 2011; Roy et al. 2009).

To deal with processes that produce more than one product (e.g., algal meal and oil), it is important to partition all inputs and outputs (other than products, e.g., emissions) to the co-products under study. Several types of allocation already exist but the choice of allocation is to a certain extent subjective and can have a drastic influence on total life cycle impacts. Therefore, according to ISO 14041, wherever possible, allocation should be avoided (Ekvall and Finnveden, 2001). As a result, system expansion was given a prominent

place in LCA. Performing a system expansion in relation to co-products is exactly to identify how the production volume of the processes will be affected by a change in demand for the product of interest (functional unit of the LCA). Nevertheless, expanding the product system to include additional functions related to the co-products is not always feasible (Weidema, 2001). System expansion may involve processes that also generate multiple products, i.e. it would involve an unending regression. Another example is when a by-product does not substitute for another alternative product, system expansion may be regarded as incompatible with the requirement that compared systems must have identical functions. In those cases, allocation cannot be avoided and one has to search for the allocation parameter that best reflects the physical relationships (mass, volume, energy or exergy value, etc.) between the environmental burdens and the functions of the products. Where such physical causal relationships alone cannot be used as the basis for allocation, the allocation should reflect other relationships between the environmental burdens and the functions e.g., economic allocation (Ekvall and Finnveden, 2001).

As a result, two types of LCAs are described in the literature: attributional LCA (ALCA) and consequential LCA (CLCA) (European Commission, 2009). ALCA is defined by its focus on the environmental burdens (average data) that are associated with the life cycle of the good and/or service produced within the system. The environmentally relevant physical flows of a past, current or potential future product system are described. The CLCA is a more market-oriented approach and is defined by its goal to identify the environmental consequences of changes that are based on a decision. A CLCA ideally includes marginal data to be able to include the marginal technologies that contribute to the environmental consequences of a change (Ekvall et al., 2016). Allocation is typically avoided through system expansion by using substitution (Finnveden et al., 2009). Because it is not convenient to know which processes are affected by a certain change in the time and/or space, the CLCA concept is usually more complex than attributional LCA. Moreover, the results obtained are highly sensitive to the assumptions that are made, which can result in a poor analysis (Sokka and Soimakallio, 2009). The choice of ALCA or CLCA should reflect the underlying goals and objectives of the study.

The LCI step includes finding information on material and energy requirements, as well as emissions and wastes associated with a product or service. Often a process flowchart is constructed to allow for a better interpretation of the value chain. Furthermore, additional LCI of the background processes is gathered, available in LCA databases such as ecoinvent.

The third phase of life cycle assessment aims at understanding and evaluating the magnitude and significance of the potential environmental impacts of a product system. Several LCIA methods are developed during recent years, either focusing on emissions to the environment (e.g., IPCC 2007) or resources extracted from the environment (e.g., ecological footprint, Wackernagel et al., 2005). However, there are also methods developed in an attempt to account for both emissions and resources (e.g., RECIPE, Goedkoop et al., 2009).

Because the use of LCA encourages preventative and proactive environmental management rather than reactive end-of-pipe approaches, and because increasing resource efficiency is a major challenge in our everyday life, especially the LCA methods that account for resource use throughout the life cycle are of interest lately. Several resource accounting methods (RAM) are already developed for application within the LCA framework. In order to provide results in single score indicators, the resources are usually represented in common units such as kg or MJ (Swart, 2015) because the efficiencies are often based on thermodynamic laws (mass, energy, exergy, emergy). A summary of RAMs can be found in the work of Huysveld et al. (2015) and Swart et al. (2015). Examples of such impact assessment methods are the Cumulative Energy Demand (CED), the Solar Energy Demand (SED) and the Cumulative Exergy Extraction from the Natural Environment (CEENE). The latter method is based on thermodynamics through quantification of resources by their amount of exergy, i.e. it is a resource footprint which detects and quantifies all exergy taken from the environment to produce the final product. This method is recommended as the most appropriate one of the available thermodynamic resource indicators for accounting and characterizing resource use, mostly through including an approach for assessing land occupation (Liao et al., 2012).

2.2 LCA of algae production: state-of-the art

The depletion of world oil supplies in combination with the increase of GHG emissions in the atmosphere as a result of burning fossil fuels has stirred interest in sustainable energy and products. As a result, the Renewable Energy Directive has been established as an overall policy for the production and promotion of energy from renewable sources in the EU. At least 20% of the total energy needs within the EU should be fulfilled with renewables by 2020. Many crops such as corn and sugar cane are currently being used as a feedstock for biofuel production, having a net energy ratio (NER) greater than one, i.e. the (fossil based) energy required to produce the fuel over its life cycle is less than its energy content (Duffield et al., 2006). Though, producing these biofuels at large scale cannot be achieved in a sustainable way because food security is compromised due to the competition of crops for arable land. Consequently, algae are stated to be a promising biomass for several purposes and applications. To identify and evaluate the environmental burdens associated with algae production, an environmental LCA should be performed. Algal production processes have undoubtedly an impact on the environment, especially related to energy consumption and atmospheric emissions (Aitken and Antizar-Latislao, 2012).

Various LCA studies on the sustainability of microalgae production have been published (e.g., Lardon et al., 2009; Jorquera et al., 2010; Soratana and Landis, 2011; Rocco et al., 2015; Grierson and Strezov, 2012; Singh and Olsen, 2011). Most of these studies focused on quantifying the impact of microalgal-based biofuel production on the environment, because algal biofuel is believed to be one of the biofuels for the future in view of its potential to replace depleting fossil fuels (Lardon et al., 2009; Chisti, 2008). However, several environmental sustainability assessments show that in most cases, producing fuel from algae (especially when cultivated in closed PBRs) has a negative energy balance, meaning that the energy demand to produce the biodiesel exceeds the energy it generates (Lardon et al., 2009; Stephenson et al., 2010; Khoo et al. (2011); Udom et al., 2013). For example, the study of Pragya and Pandey (2016) investigates the net energy balance of biodiesel produced from algae in open raceway ponds and flat-plate airlift PBRs (both wet and dry routes) and the results are remarkable: even the best possible route (open ponds, dry route) has an energy use that is almost 5 times more than what can be produced. In

addition, CO_2 is emitted throughout the life cycle rather than being sequestered, i.e. there is a net CO_2 emission. The negative environmental balance, the high costs related to the production and processing and the relatively low market price of the commodity (fuel) clearly make (micro)algae production solely for energy purposes unsustainable in the short term (Hafting et al., 2015; Pragya and Pandey, 2016). However, if one accounts for coproducts, recycling of nutrients and absorption of CO_2 from flue gases, biodiesel production from algae actually shows greater long-term potential than terrestrial biofuels such as corn ethanol (Liu et al., 2012; Gnansounou and Raman, 2016).

Another point of attention is the fact that LCA studies focusing on seaweed production and processing are published to a much lesser extent than studies on microalgae. Furthermore, the environmental footprint of algae production targeting higher value products (e.g., nutraceuticals and pharmaceuticals) rather than commodity production, whether or not in combination with pollution treatment, is poorly addressed. Additionally, data for the published LCA studies are generally collected from literature or are based on small-scale lab trials, which add to uncertainty. Due to the lack of data from large scale operative plants, the studies are often based on conceptual designs, models, extrapolations and assumptions and in the best case on pilot scale data (Collet et al., 2015). Moreover, inconsistent system boundaries, functional units and co-product allocation methods hinder a fair comparison of the results. A meta-analysis of several scientific LCA publications showed contradictory results, which makes the environmental benefit of algae production rather unclear and somewhat speculative (AquaFUELs, 2009).

LCA has evolved significantly over the past three decades to become a valuable decisionsupport tool that can be used by manufacturers, suppliers, customers, policy-makers and other stakeholders. Yet, environmental sustainability assessment is a holistic and complex task that still needs elaboration in many directions (Guinée et al., 2011). One LCA challenge is related to the fact that algae can be cultivated on marginal, non-arable land or in the sea, which is an enormous advantage because (fertile) land is today a scarce resource. Nevertheless, accounting for the environmental benefit of using marginal land instead of fertile land used by other biomass types (e.g., food crops) or the use of sea surface is not aphoristic. The assessment of terrestrial land use (or land occupation) has gained wide attention already in LCA but efforts still have to be made to account for the environmental impact of different types of land use in a consistent way. Several questions arise: should we account for resource depletion related to land use or focus of the impact on ecosystem functioning? What is the best way to grasp these impacts, i.e., what kind of data do we use to calculate these impacts? Would it be feasible to develop one method that accounts for all land use impacts or do we let experts develop LCA methods that account for specific land use impacts? These questions still need to be answered and a more detailed guideline on global land use impact assessment is required. Additionally, accounting for the impact of using sea surface (e.g., seaweed farming) in LCA is even a more difficult task because of the complexity of the marine environment. Until now, LCIA methods that account for the use of sea surface are lacking. Moreover, to describe region-specific impacts, the LCA methodology still has points requiring elaboration and improvement to integrate spatial and temporal differentiation.

Chapter 3 Objectives and overview of the study Chapters 1 and 2 (Part I) illustrate the enormous potential of micro- and macroalgae for several industrial sectors. As the production is a rather young technology (especially in Europe), it faces severe problems regarding costs and environmental pressure because of the energy intensive processes along the production chain. Breakthroughs that may allow algae to play an essential role in meeting future demands will include the selection of highly productive species with specific components of interest and improved cultivation and harvesting methods. To be used in a sustainable way in the long term, it seems promising to produce algae for higher value applications (e.g., feed, cosmetics) in a biorefinery setup, ideally in combination with waste stream mitigation. Thus, although there is already great interest in algae production, it seems that more research and cooperation is necessary to provide full scale algae production in a sustainable way. Apart from efforts necessary in the field of algae production, also several aspects of the LCA methodology may be improved to better quantify the environmental footprint of algalbased products. One of the improvements involves quantifying the impact of occupying surface area, both marine sea surface and terrestrial land, and the impact of consuming the natural resources provided by land and oceans. Until now, the benefit of cultivating aquatic biomass on marginal land or nearshore, compared to most crops that require fertile land, has been poorly addressed in LCA.

In this context, the objectives of this PhD dissertation are two-fold (chapter 3):

1) Gaining insight in the environmental footprint of microalgae production for higher value applications, and more specific as a feed ingredient, on the one hand produced in an innovative cultivation system and on the other hand in a biorefinery. Both case studies consider waste stream mitigation.

2) Further development of LCIA methods to better account for the impact of occupying terrestrial land and marine sea surface during algae production to make a more fair comparison with the footprint of alternative terrestrial plants.

To realize these objectives, different studies have been performed. The complete outline of this dissertation is schematically shown in Figure 5.

To address the first objective, **Chapters 4 and 5** (*Part II*) represent case studies on the environmental sustainability of microalgae production for feed applications, on the one hand to be used in aquaculture and on the other hand produced as a livestock feed ingredient. In both chapters, a comparison with land-based alternatives has been made. The environmental resource footprint of dried microalgal biomass versus a traditional fish feed was calculated and the impact of protein-rich microalgal meal produced to feed livestock was compared with the impact of importing Brazilian soybean meal.

In Part III of this PhD dissertation, focus was put on an improved quantification of the environmental impact caused by anthropogenic surface use. Chapter 6 deals with the possible impact of using the marine environment, as this was not quantifiable before in LCA. To allow for region-specific impacts, exergy based spatial (and temporal) characterization factors were calculated for ocean areal occupation. In relation to algae production, a better accounting for the use of marine resources is extremely relevant when considering nearshore or offshore seaweed cultivation. To demonstrate the new methodology, the environmental footprint of two seaweed production systems in North West Europe (NWE) was analyzed. In addition, chapter 7 consists of an overview of different types of land use activities and points out the possible effect on natural landbased processes and stocks and funds that can be altered due to land use. Several indicators are already available to be used in an LCA framework to quantify certain land use impacts. However, in this chapter, two enhanced proxy indicators are proposed to better assess the impact of occupying land on ecosystem health. Again, exergy-based spatially differentiated characterization factors are presented for several types of land use (e.g., pasture land, urban land) in different countries. This allows a more fair comparison between marginal land use for algae production and fertile land use for cultivating terrestrial crops. Advantages and drawbacks are mentioned for each proposed indicator in both chapters.

Chapters 8 and 9 (*Part IV*) are the conclusive and perspective chapters, in which overall conclusions related to the feasibility of algae production (in Europe) are drawn and recommendations for future research are mentioned.



Figure 5 Outline of this PhD dissertation

PART II

Environmental sustainability analysis of microalgae production systems for feed applications

Chapter 4

The environmental sustainability of microalgae as feed for aquaculture: A life cycle perspective²

² Redrafted from

Taelman, S.E., De Meester, S., Roef, L., Michiels, M., Dewulf, J. (2013). The environmental sustainability of microalgae as feed for aquaculture: A life cycle perspective. Bioresource Technology 150, 513–522.

4.1 Introduction

Initially, microalgae research focused mainly on the production of biodiesel, bioethanol and biogas from algae as a response to the growing demand for fuel and the depletion of fossil resources (Lardon et al., 2009; Chisti, 2008). A few studies have been published on biohydrogen generated from algae-utilizing bacteria and the production of syngas or bio-oil. However, current research shows that cultivating microalgae solely for energy purposes is not sustainable yet (Stephenson et al., 2010 and Udom et al., 2013). Because algae are such rich type of biomass, containing large amounts of essential carbohydrates, proteins, lipids, vitamins and antioxidants, the potential of algae to be used in other sectors is huge. The only way to offset the commercial and environmental costs of cultivating and processing algae would be to guarantee production of high quality end-products.

One of the sectors that show a strong interest in algae is the aquaculture industry. The rising demand for seafood can no longer be satisfied by capture fisheries alone, which results in a rapidly expanding aquaculture production (García-Ortega et al., 2016). According to FAO, 42% of total fish production came from aquaculture in 2012, and in 2030 it is expected to increase to 50% (FAO, 2013, 2014). The feeding fish to fish principle used nowadays in this sector is unsustainable because more than 1 kg fish is needed to produce 1 kg of carnivorous farmed fish. Small pelagic species, such as Peruvian anchovy, are cocaptured and subjected to several processes to produce fish oil and fish meal, which are mainly used as feed in aquaculture systems. Researchers around the globe are concerned because aquaculture production is expected to continue to rise in the short term. This will result in increased fishing pressure on wild stocks to supply both fish meal and fish oil, which threatens the sustainability of the species in question. Prices for these fish-based products are already increasing, which has sparked a search for alternatives to sources of omega-3 and omega-6 (long-chain) poly-unsaturated fatty acids (Shepherd, 2013). Algae biomass could be a workable alternative to replace fish meal and fish oil in aquaculture diets because algae are the preliminary food source in the rearing of all stages of marine bivalve molluscs (clams, oysters, scallops), the larval stages of some marine gastropods (abalone, conch), larvae of several marine fish species and penaeid shrimp, and zooplankton (Muller-Feuga, 2000). Moreover, algae can potentially devastate the

contamination by bacteria that attack fish in aquaculture farms because microalgal fatty acids containing 10 carbon atoms or more can induce lysis of bacterial protoplasts (Guedes et al., 2011). Also, the contents of carotenoids are important in the aquaculture feed diet for e.g., salmon or trout to acquire their characteristic red color; without such a color, a lower market value will result (Guedes and Malcata, 2012). Commercial-scale production of microalgae would reduce both the cost and ecological impact of intensive fish farming (Muller-Feuga, 2000). Because of the large short term market potential of aquaculture, cultivating algae for fish feed applications has the potential to become profitable in the near future.

As described in section 1.2.3, there are two main systems available to cultivate microalgae, namely open ponds or closed PBRs. In 2009, Michiels unveiled the patented ProviAPT (Proviron Advanced Photobioreactor Technology). This innovative system has the advantages of closed reactors yet avoids the drawbacks of open systems (e.g., contamination, evaporation) and closed systems (e.g., scaling up). It is a plastic bag (12m²) filled with water. Each bag, which rests on the ground, contains 35 embedded plastic panels where the algae grow. This yields a reactive surface of $7m^2$ (Michiels, 2009), as visualized in Figure 6. Water and nutrients are pumped into one side of the panels and algae are harvested on the other side via an overflow system. This system is relatively inexpensive to build (< $\leq 10/m^2$) because the structure is constructed entirely of plastic and the production can be automated.

This study is to examine the entire production process in an industrial setup and, via two hypothetical upscaling scenarios, to optimize the configuration for a more sustainable algae cultivation. Three algal production scenarios were studied: 1) the pilot setup of *Nannochloropsis sp.* cultivated in 20 ProviAPT reactors was based on actual production runs (pilot 2012 covers 240m²), 2) a realistic but hypothetical upscaling of pilot 2012 including recycling and more efficient use of equipment (the pilot 2013 scenario covers 1320 m²) and 3) a further hypothetical upscaling using waste streams and a more efficient processing equipment in a warmer climate such as in Spain (first production scenario 2015 covers 2.5 ha).



Figure 6 (A) picture of ProviAPT system (2 bags); (B) schematic representation (top view) of one ProviAPT bag (in meters). Total surface area of the reactor bag is 12m², and the reactive surface area 7m².

For all three scenarios, an exergy analysis (EA) and LCA was performed. The exergy concept was applied because exergy quantifies the ability to cause change and is not conserved, in contrast to energy, which exposes the inefficient processes (Dewulf et al., 2008). During the exergy analysis, the exergy consumption as well as the exergy efficiency of the different subprocesses (process level) and of the entire foreground production system (gate-to-gate) was determined. Furthermore, a cradle-to-gate LCA has been performed because of the increasing awareness of the possible impact associated with the full chain of products, processes and services. The resource consumption of the entire product life cycle was determined using the Cumulative Exergy Extraction from the Natural Environment (CEENE) method (Dewulf et al., 2007a). In addition, the impact on climate change was assessed using the IPCC 2007 method (IPCC, 2007). This study had two main objectives: 1) to examine different aspects of the ecological sustainability of the production of microalgae in the ProviAPT cultivation system and 2) to determine the potential of microalgae production as fish feed application and to make a comparative study of the environmental impact of algae based fish feed versus traditional fish feed.
4.2 Materials and methods

4.2.1 Description of the process

In 2012, *Nannochloropsis sp.* was cultivated in 20 ProviAPT PBRs (240 m²) at a production site near Antwerp, Belgium. The PBR consisted of a large transparent bag (surface area 12 m²) containing embedded plastic panels. The surface area under the panels measured a total of 7 m² ('productive area'). Nutrients and CO_2 -enriched air were injected (semi-)continuously into the panels and the microalgae were harvested via an overflow system. The 5000 liters of water surrounding the panels buffered the temperature and provided the necessary support for the reactor. During winter, the reactor was kept at constant temperature (22°C) using waste heat from an electricity generator by circulating the water in the outer reactor bag across a heat exchanger (Roef et al., 2012).

The cultivation medium was prepared in the medium tank (Figure 7, process A) where nutrients, salt and water were mixed to the desired concentration. Subsequently, the medium was pumped into the PBR bags (process B). The algae obtained their carbon from CO_2 bottles via a fan. Based on the CO_2 consumption, air was released from the PBR at overpressure and the oxygen produced was automatically removed to avoid toxic concentrations. The harvested algae (2.1 g L^{-1}) were transported to an aerated flat plate microfiltration membrane (process C). The retentate of 3% DM was pumped to a bowl separator centrifuge where the algae biomass was concentrated to 19% DM (process D). The last step was to freeze-dry the algae to 95% DM (process E). The choice to freeze-dry the algae was dictated by specific customer demands about the quality of the product sold. All processes used electricity sourced from the Belgian electricity grid. In this setup, the final product was 17 tonnes of dried algal biomass per hectare per annum (based on the total surface area of the reactor bags, Figure 6). All the algae produced was sold as aquaculture feed. Approximately 80% of all data from the pilot facility of 2012 were collected directly on-site, while the other necessary data were computed through mass and energy balancing or were found in literature (less than 5% of all data).



Figure 7 Process flow scheme microalgae production chain (pilot 2012, scale 240 m²). Data inventory per MJ_{ex} dry matter algal biomass. Overall there are five distinct subprocesses within the foreground system (indicated by the red dotted line): nutrient mixing (process A), cultivation *Nannochloropsis sp.* (process B), membrane separation (process C), centrifugation (process D), freeze drying (process E).

This study also assessed the raw material consumption and impact on climate change of two hypothetical, larger-scale scenarios. Table 2 shows the differences in the inventory between the first pilot setup and the two hypothetical scenarios. Most of the data for the latter scenarios were obtained through sensitivity analysis based on the first pilot plant as well as consultation with the industry. These hypothetical scenarios were expected to result in improved algae production facilities based on realistic scale-up dimensioning.

In 2012, it was envisaged that the first and second hypothetical scenarios would start in 2013 and 2015, respectively (Michiels M., personal communication). The area foreseen for algae production in the pilot 2013 scenario was 1320 m² in contrast to 240 m² (2012). The permeate of process C and a large part of the centrate of process D were recycled. An

additional filtration step was identified as necessary to remove micro-organisms from the centrate. The retentate (cake) of this step will be discharged. In this scenario, the CO_2 input came from bottled gas. Due to upscaling, the efficiencies of the fan and the freeze dryer (operation at full capacity) were projected to increase. The biomass yield was similar to that of the first pilot plant, i.e., 17 tonnes DM per hectare per year.

A first production scale scenario was also investigated with 2.5 hectares of land covered with PBRs. Our production scale scenario revealed the potential for producing 55 tonnes DM ha⁻¹ year⁻¹. In this scenario, the algae cultivation and processing were no longer located in Belgium, but at a location with higher solar irradiation and warmer climate (e.g., Spain). Under those climatic conditions, it was no longer necessary to use an industrial waste heat source during wintertime, but the circulation pump was still used for cooling the reactors in summer. In this scenario, the fan reached its highest efficiency, around 80% (UNEP, 2006a). CO_2 was theoretically sourced from industrial exhaust gases. The size of the PBRs was optimized, with the 'productive surface' approximately 21 m² and the entire reactor surface area approximately 26 m² per bag. The average standing stock (i.e., harvested) cell densities were estimated to double to 5 grams DM algae per liter instead of 2.1 g L⁻¹ due to higher solar irradiation and further optimization of reactors and algae culture (Michiels M., personal communication). The effluent of the PBR (2.7% DM; algae, salt, nutrients) was pumped to a filtration unit where it was concentrated for the first time, resulting in a retentate of 4.6% DM. The permeate was sent back to the medium tank and the retentate was brought to an industrial hydrostop separator operating at less than 1 kWh m⁻³ (Piek, 2012). The concentrate of the centrifuge attained a dry matter (DM) content of 28% (Michiels P. (GEA Westfalia), personal communication). Using a membrane treatment to remove micro-organisms, the centrate was also recycled. The concentrated stream was then pumped to an industrial gas fired drum dryer commonly used to dry organic streams, e.g., soybeans (Sander and Murthy, 2010). In this process, the algal biomass was dried to 95% DM to facilitate comparison with the other scenarios.

	Pilot 2012	Pilot 2013 scenario*	First production scale 2015*	
Scenario (generic)				
Scale	240 m ²	1320 m ²	2.5 ha	
Location	Belgium	Belgium	Spain	
Recycling of permeate and centrate	No	Yes, extra filtration on centrate	Yes, extra filtration on centrate	
(process C and D)				
Photobioreactor				
Area PBR	12 m ²	12 m ²	26 m ²	
Reactive area PBR	7 m ²	7 m ²	21 m ²	
Photosynthetic efficiency	2% on 7m ²	2% on 7m ²	3% on 21m ²	
	= 1.2% on 12 m ²	= 1.2% on 12 m ²	= 2.4% on 26 m ²	
Areal productivity	4.59 g (m ² d) ⁻¹	4.59 g (m² d)-1	15.1 g (m ² d) ⁻¹	
Volumetric productivity	0.53 g (L d) ⁻¹	0.53 g (L d)⁻¹	1.25 g (L d) ⁻¹	
Efficiency fan	25%	35%	80% ^(a)	
Efficiency circulation pump	11%	11%	80% ^(b)	
Source CO ₂	bottled	bottled	Flue gases	
Heat	200 W m ⁻²	200 W m ⁻²	No extra heat necessary	
Centrifugation				
Туре	Bowl centrifuge	Bowl centrifuge	Hydrostop centrifuge	
Electricity consumption	15 kWh m⁻³	3.5 kWh m⁻³	0.95 kWh m ^{-3(c)}	
Drying				
Туре	Freeze dryer	Freeze dryer	Drum dryer	
Electricity consumption	59 kWh kg⁻¹ DW	13 kWh kg $^{-1}$ DW	/	
Natural gas consumption	/	/	0.98 kWh kg ⁻¹ H ₂ O _(vapour) ^(d)	

Table 2The main differences in the basic inventory of the three scenarios.

^(a) based on document UNEP, 2006a

^(b) based on document UNEP, 2006b

 $^{\rm (c)}$ Piek, 2012 $^{\rm (d)}$ Sander and Murthy, 2010

* Hypothetical upscaling scenarios

4.2.2 Exergy analysis: process level and gate-to-gate level

Exergy is a thermodynamically based measure and is defined as the maximum amount of work obtainable from a system or resource, as it is brought to equilibrium with a reference environment (as defined by Szargut et al. (1988) with its reference temperature T_0 (298.15 K), pressure P_0 (1 atm) and composition) through reversible processes. Exergy is not subject to conservation rules; exergy can be destroyed due to irreversibilities during any process, i.e. the final exergy embodied in delivered work, heat, (by)products and waste is not equal to the initial exergy content of the resources (Dewulf et al., 2008). The use of the unit 'exergy' instead of 'energy' has some advantages: (1) not only the quantity but also the quality of a resource can be assessed, (2) all of the resources can be expressed in the same unit; this in turn facilitates interpretation and comparison of results (Dewulf et al., 2008), and (3) because exergy is not conserved, it exposes inefficient processes, which indicate the loss of work potential. An important strategy for improving the sustainability of the real processes is to reduce the rate of exergy loss, i.e., entropy production, or to increase the exergy efficiency (De Meester et al., 2009; Dewulf et al., 2008).

According to Szargut et al. (1988) and Kotas (1995), the exergy of a system is split up into four parameters: kinetic exergy, potential exergy, chemical exergy and physical exergy. However, potential and kinetic exergy are equivalent to potential and kinetic energy, respectively, and are normally neglected (Romero and Linares, 2014). On top of the relevant physical and chemical exergies, Dewulf et al. (2008) defined other types of exergy sources such as electric exergy, nuclear exergy and (solar) radiation exergy. The calculation of the total exergy of a product or process within this study was based on several exergetic parameters (Equation 1 (Eq. 1)): physical exergy (Ex_{ph}), chemical exergy (Ex_{e}) and radiation exergy (Ex_r). The reference environment applied for this study has been defined by Szargut et al. (1988) with a reference temperature of 298.15 kelvin and a reference pressure of 1 atmosphere.

$$Ex_{tot} = Ex_{ph} + Ex_{ch} + Ex_{e} + Ex_{r}$$
(1)

Physical exergy

The physical exergy is the maximum useful work obtained by passing the unit of mass of a substance at the generic state (T, p) to the environmental (T_0 , p_0) state through purely physical processes. For the calculation of the physical exergy of the warm liquid flow, equation 2 was used (Szargut, 2005) at constant pressure ($p = p_0$). In this equation m is the mass (kg), c_p the heat capacity (kJ kg⁻¹ K⁻¹), T the temperature of the substance (K) and T_0 the reference temperature (K).

$$Ex_{ph} = m \cdot c_{p} \cdot [(T - T_{0}) - T_{0} \cdot \ln (T/T_{0})]$$
(2)

Chemical exergy

To calculate the standard chemical exergy (kJ mol⁻¹) of components, Tables 1 and 2 in the SI of Szargut et al. (1988) were available for retrieval of the chemical exergy value of many organic and inorganic compounds, including the corresponding reaction from their reference components. If an organic substance was not listed but the molecular formula was known, the chemical exergy was calculated on the basis of group contribution, as proposed by Shieh and Fan (1982). Table 3 in the SI of Szargut et al. (1988) listed the standard chemical exergy (kJ mol⁻¹) for most of the common chemical groups. For non-listed inorganic substances, the chemical exergy content was calculated using the Gibbs free energy method (Kotas, 1995). To determine the chemical exergy of the algal biomass, the macronutrient method was used where the composition of the various molecules such as carbohydrates, proteins, lipids, ash and water were determined and their exergy content was calculated by using one of the above-mentioned methods (De Vries, 1999).

The chemical exergy content of a gas mixture is always lower than the sum of the exergy of the constituent components, which was mainly relevant when calculating the exergy of a nutrient mix. Therefore, the mixing exergy value was always a negative number. It was calculated by means of equation 3 where R is the gas constant 8.31 J mol⁻¹ K⁻¹, T₀ the reference temperature (K), χ_i the mole fractions of the various components in the mixture and γ_i is the activity coefficient. For ideal mixtures this factor is equal to 1. This number was applied in this study.

 $Ex_{mix} = R \cdot T_0 \cdot \sum \chi_i \cdot ln(\gamma_i \cdot \chi_i)$

(3)

Electrical exergy

Electricity is a high quality form of energy and therefore the energy value was equated to its exergy value (Wall, 1997).

Radiation exergy

To calculate the radiation exergy, equation 4 was used. In this study, the radiant exergy of the sun (5800 K) was calculated. Upon insertion of that value, the simplified equation 5 was obtained and was used to determine the photosynthetic yield of the algal biomass produced. The radiation energy value for the ProviAPT installation located in Belgium was set at 2.69 kWh m⁻² day⁻¹ and for Spain 4.48 kWh m⁻² day⁻¹ (Huld et al., 2012).

$$Ex_{r} = [1 + 1/3 \cdot (T_{0}/T)^{4} - 4/3 \cdot (T_{0}/T)] \cdot energy$$

$$Ex_{r} = 0.9326 \cdot energy$$
(4)
(5)

Efficiencies and ratios

For each subprocess at each scenario, an exergy balance was prepared and used to assess the exergy efficiency Ψ_a of each process. At the gate-to-gate level, the global efficiency Ψ_a was calculated, in addition to the rational efficiency Ω_a where only the exergy available in the algal biomass was taken into account as output. Because sunlight is often considered a free resource, the ratios Ψ_b and Ω_b were also computed without this input.

$\Psi_a = (\sum Exergy outputs) / (\sum Exergy inputs)$	(6)
Ψ_{b} = (Σ Exergy outputs)/(Σ Exergy inputs - exergy contained in sunlight)	(7)
$\Omega_{_{ m a}}$ = (Exergy algal biomass)/(Σ Exergy inputs)	(8)
$\Omega_{ m b}$ =(Exergy algal biomass)/(Σ Exergy inputs - exergy contained in sunlight)	(9)

In addition to the efficiencies and ratios defined above, the photosynthetic exergy yield μ of *Nannochloropsis sp.* was calculated according to equation 10. Furthermore, the overall exergy breeding factor (BF_{ex}) was assessed by equation 11, showing how many renewable resources were bred from non-renewable resources across the entire production chain (Dewulf et al., 2005).

μ= (Exergy algal biomass)/(∑Exergy sunlight)		
overall BF _{ex} = (Exergy algal biomass)/(Σ Exergy in non-renewable inputs)	(11)	

From the cradle-to-gate perspective, only net nutrient rich water, net CO_2 and electricity use belonged to the non-renewable resources for the pilot 2012. For the first production scale scenario (2015), the drying process used natural gas as a non-renewable input but the CO_2 from flue gases was a waste stream from the technosphere.

4.2.3 LCA: cradle-to-gate level

LCA is used to determine the potential environmental impact of microalgae production. The different steps of an LCA were executed according to the recommendations of the ISO 14040 and 14044 standards (ISO, 2006a,b), as explained in section 2.1.

Goal and scope

The environmental sustainability of the production and processing of *Nannochloropsis sp.* as a biomass raw material was assessed on the basis of energy and mass flow analysis as well as a cradle-to-gate LCA. In other words, a cradle to gate (exergetic) life cycle has been performed to determine the environmental sustainability of the entire process. A comparison was made with the resource consumption and carbon footprint of a traditional reference fish feed production (17.31 MJ_{ex} kg⁻¹) used to feed pangasius juveniles (Huysveld et al., 2011; Huysveld et al., 2013) to obtain more insight into the environmental competitiveness of microalgae in this market. The ingredients of the traditional feed were fish meal (8%), fish oil (1%), poultry by-product meal (2%), wheat grains (19%), wheat bran (10%), rice bran (35%), soybean meal (25%) and some additives such as vitamins, probiotics and medicines. In general, this feed consisted mainly of terrestrial biomass resources (Huysveld et al., 2013).

The functional unit chosen for the data inventory and the impact assessment methods has been proposed as 1 MJ exergy embodied in dry *Nannochloropsis sp.* biomass (23.07 MJ_{ex} kg⁻¹ DM) because exergy is stated to give an indication on the amount of useful energy available for the fish metabolism, as such addressing the bioavailability aspect of nutrition (Sturtewagen et al., 2016). The red dotted line in Figure 7 (page illustrates which processes are included within the foreground system. A cradle-to-gate boundary analysis was applied that included all products and processes in the background, i.e., the supply chain. The processes that were negligible (5% or less of the overall environmental impact) were transport of nutrients and CO_2 to the algae culture and transportation of dry biomass after processing. According to the outcome of preliminary calculations, the contribution from infrastructure seemed to be minor and was therefore excluded from the foreground system boundaries. With respect to emissions from the foreground system, only CO_2 was taken into account. Other potential emissions were not tracked.

Life-cycle inventory

The inventory for the background processes was selected from the ecoinvent version 2.2 database (Frischknecht and Rebitzer, 2005). The ecoinvent processes listed in Table 3 were used to model the production chain of the inputs. In this study, the environmental impact was not allocated because there was only one final product produced, namely dried algal biomass (95% DM).

Product/process	Unit	Pilot 2012
Calcium chloride, CaCl ₂ , at plant	kg	2,25E-02
Iron sulfate, at plant	kg	3,40E-04
Sodium chloride, powder, at plant	kg	4,33E-01
Magnesium sulfate, at plant	kg	1,09E-01
Chemicals inorganic, at plant	kg	1,43E-01
Sodium carbonate from ammonium	kg	3,41E-03
Potassium chloride, as K ₂ O, at regional storehouse	kg	3,41E-02
EDTA, ethylenediaminetetraacetic acid, at plant	kg	4,58E-04
Polypropylene, granulate, at plant	kg	8,26E-09
Boric acid, anhydrous, powder, at plant	kg	5,99E-04
Carbon dioxide liquid at plant	kg	1,07E-01
Tap water, at user	kg	2,90E+01
Electricity, production mix Belgium	kWh	4,98E+00
Electricity, production mix Spain	kWh	n/a ⁽¹⁾
Land	m².year	n/a ⁽²⁾

Table 3Ecoinvent processes used to analyze the algae production system in 2012 expressed
per day and per photobioreactor.

⁽¹⁾ Electricity from the Spanish grid is used only in the first production scale scenario 2015.

⁽²⁾ The CEENE method considers the solar exergy deprived from the natural ecosystems for all land surface occupation categories. In total, $681.4 \text{ GJ}_{ex} \text{ ha}^{-1} \text{ yr}^{-1}$ is withdrawn from nature.

Impact assessment

To determine the resources used in the life cycle of the production of dry algal biomass, the CEENE methodology was used (Dewulf et al., 2007a), as explained in section 2.1. In the CEENE method, natural resources are divided into eight categories: renewable resources, fossil fuel resources, nuclear resources, metal ores, minerals, water resources, land resources and atmospheric resources. The renewable resources category includes wind power, geothermal energy and hydropower whereas land use includes land dependent (renewable) biomass (Dewulf et al., 2007a). For European climate conditions, the average solar irradiation is 2.78 kWh m⁻² day⁻¹. Considering the exergy to energy ratio of 0.9327 and plants' ability to metabolize a maximum of 2% of the incoming solar radiation, the characterization factor for land use is 68.14 MJ_{ex} m⁻² year⁻¹ (Dewulf et al., 2007a; Liao et al., 2012; Rugani et al., 2011). Additionally, the carbon footprint method IPCC 2007 was used to quantify the impact on global warming by means of determining the $CO_{2,eq}$ emissions (IPCC, 2007).

4.3 Results and discussion

4.3.1 Exergy analysis: process level

An exergy analysis was made of the algae pilot scale facility of 2012 and the forecast scenarios for 2013 and 2015. The chemical, physical, electrical and radiation exergy of all material and energy flows of each subprocess were calculated. Table 4 gives the exergy efficiencies at process level for each scenario.

		Hypothetical	Hypothetical
	Pilot 2012	Pilot 2013	First production scale 2015
Mixing process	72.6	78.6	96.3
Cultivation	2.62	2.64	3.58
1 st membrane filtration process	99.6	99.6	99.7
Centrifugation	84.8	97.0	99.5
2 nd membrane filtration process	/	99.3	99.3
Drying	9.90	33.3	72.8
$\overline{\Psi_{a}}$	2.36	1.29	2.43
$\Psi_{ m b}$	14.5	15.6	90.6
Ω_{a}	1.00	1.10	2.28
$\Omega_{ m b}$	6.18	13.2	84.9

Table 4 Exergy efficiencies at process level and exergy ratios Ψ_{a} , Ω_{a} , Ψ_{b} and Ω_{b} at gate-togate level of the three scenarios, %.

Pilot plant (2012)

In pilot plant 2012, 0.47 MJ_{ex} electricity per MJ_{ex} DM was used to mix the different ingredients. This accounted for 27.4% of the exergy input. As can be concluded from Table 4, only the exergy of the electricity used was lost in the first subprocess. Similarly, in the membrane separation, the centrifugation and the freeze drying process, the exergy of the electricity used was dissipated and thus not captured in their output products. This resulted in Ψ_a efficiencies of 99.6%, 84.8% and 9.90%, respectively, which revealed that the drying process in particular was very energy intensive. At first glance the cultivation process seemed very exergy inefficient (2.62%) due to the large input of solar exergy of which only a small fraction was photosynthetically converted. However, when sunlight was considered as a free resource, the exergy ratio Ψ_b of the cultivation system was 37.3%. The electricity needed to operate the fan and the circulation pump was identified as important. The photosynthetic exergy yield μ derived from this process amounted to 1.20%.

Hypothetical: pilot plant (2013)

The 2013 hypothetical scenario was more efficient than the pilot plant 2012, as can already be seen in the first subprocess. The nutrient mix had the same exergy content as in 2012 but less electricity was consumed for the mixing process, which reduced the total exergy input. The cultivation step had a similar exergy efficiency Ψ_a as in 2012. When the exergy content of the sunlight was not taken into account, the exergy ratio Ψ_b became higher in 2013 (45.8%) due to a lower electricity consumption of the fan in this scenario. The photosynthetic exergy yield μ remained 1.20% and the exergy efficiency Ψ_a of the membrane separation step remained the same. A membrane filtration step was included in this scenario to meet the need for cleanup before reuse, but this separation process had a high exergy efficiency (99.3%). Only the exergy content of the necessary electricity was lost. The exergy efficiency Ψ_a of the centrifugation step increased (97.0%) because of a more efficient use of electricity. For the same reason, the freeze-drying step performed better by a factor of 3.

Hypothetical: first production (2015)

In our hypothetical scenario of a first production scale setup 2015, the mixing process was exergetically more efficient than the 2012 and 2013 scenarios because less electricity was used. The cultivation process also scored better in terms of exergy efficiency, especially because increased exergy output (more algal biomass, water, salt, vitamins, nutrients and oxygen per PBR because the PBR bags were bigger than in the previous hypothetical scenario and the actual pilot plant) in combination with decreased electricity consumption for the fan and the circulation pump per PBR. In addition, no heating was required. Despite higher levels of solar radiation available per m² cultivation bag, the exergy efficiency was still better than in the previous scenario. This can be attributed mainly to the relatively higher microalgae production: the photosynthetic exergy yield (2.40%) doubled in comparison to 2012 and 2013 because of the optimized harvesting regime and expected improvements arising from technical changes to the PBR (Michiels M., personal communication). When excluding sunlight from the calculations, the exergy ratio became higher than 2 in this scenario. This means that the exergy contained in the output biomass stream was larger than the input of 'man-made' exergy streams such as electricity production. The membrane separation process had an exergy efficiency similar to the previous scenarios (99.7%).

The input and output exergies have increased proportionally by factor 5 per day in comparison to the separation process in 2013. The exergy input was higher due to higher electricity consumption for pumping and aeration. Effluent from the PBR, with its associated higher exergy content, also entered the separation process. The exergy output increased due to larger flows with more algal biomass. In this first production scale

scenario, the hydrostop centrifuge reached an energy consumption of only 0.95 kWh m⁻³, making this process far more exergy efficient. The exergy efficiency for the membrane separation process located in the recycling stream was the same as in 2013 because the electricity consumption remained linear with the quantity of input to be processed. The electricity consumption, however, contributed only for a small part to the exergy input. The exergy efficiency was mainly determined by the exergy content of the permeate and retentate compared to the incoming centrate. For the drying process, the total energy consumption of the drying drum (hot air, natural gas) was lower than the freeze dryers in 2012 and 2013, making the exergy output to input ratio 72.8%. This represented the largest process improvement compared to the previous scenario.

4.3.2 Exergy analysis: gate-to-gate level

At the gate-to-gate boundary, an EA was made for the 2012, 2013 and 2015 scenarios. The exergy content of all streams entering and leaving the foreground system boundaries was calculated. Table 4 lists the resulting exergy efficiencies and exergy ratios for the respective scenarios.

Pilot plant (2012)

The exergy efficiency Ψ_a of pilot plant 2012, which contained 20 bags, was 2.36%, based on the comparison of exergy inputs (99.8 MJ_{ex} MJ_{ex}⁻¹ DM) and outputs (2.35 MJ_{ex} MJ_{ex}⁻¹ DM) across the full system. The rational exergy efficiency Ω_a , considering only the exergy available in the algal biomass, amounted to 1.00%. Without the inclusion of solar exergy, the ratios were 14.5% and 6.18% for Ψ_b and Ω_b respectively.

Hypothetical: pilot plant (2013)

At the gate-to-gate level of the hypothetical 2013 scenario, 91.2 MJ_{ex} per MJ_{ex} DM entered the system and only a small part could be converted into algal biomass. This resulted in a rational efficiency Ω_a of 1.10%. The global exergy efficiency Ψ_a was 1.29%. This appeared to be lower than in the 2012 scenario due to the recycling of two permeates instead of discharging a permeate and a centrate. As a result, only 1.18 $MJ_{ex} MJ_{ex}^{-1}$ DM left the system boundaries; therefore, the global exergy efficiency was lower than that of pilot 2012. In that way, it was more scientifically sound to compare the scenarios based on their rational efficiency Ω_a . When the exergy contained in sunlight was not taken into account, the Ψ_b ratio was 15.6% and the Ω_b ratio was 13.2%.

hypothetical: first production scale (2015)

In this hypothetical scenario, a total of 43.9 $MJ_{ex} MJ_{ex}^{-1}$ DM entered the foreground system boundaries and 1.07 $MJ_{ex} MJ_{ex}^{-1}$ DM left the system, making the global efficiency Ψ_a 2.43% (90.6% excluding solar exergy). The Ω_a was clearly the highest of all three scenarios: 2.28 % of all incoming exergy could be converted into algal biomass (or 84.9% when sunlight was not included). When the same drying equipment (freeze dryer) would be used with the same working efficiency as in the previous scenario, the rational exergy efficiency Ω_a would drop to 1.77%, demonstrating the importance of the more energy-efficient drum dryer.

4.3.3 Impact assessment on the basis of LCA

A cradle-to-gate LCA of the three scenarios was performed, resulting in an evaluation of the environmental sustainability of dry algal biomass production. For both the CEENE method and the carbon footprint method, the results are expressed per MJ exergy contained in 95.0% dry microalgae.

Pilot plant (2012)

Figure 8A illustrates the resource footprint of the microalgae pilot scale (240 m²) facility 2012. In total, 55.5 $MJ_{ex,CEENE}$ was extracted from the natural environment to produce 1 MJ exergy algal biomass. The biggest contribution to the resource impact was the electricity consumption for the freeze dryer (52%), followed by the electricity of the fan (23%), the input of salt (10%) and nutrients (3%), land use (3%) and electricity production for mixing (3%). Water and CO_2 inputs had only small contributions (2%) while other inputs were negligible. In general, nuclear (44%) and fossil resources (38%) were extracted most. This was attributed to the typical Belgian electricity mix, which relies mainly on nuclear power plants and natural gas to generate electricity, followed by 8% raw materials from the water and land occupation categories.

The total carbon footprint of this production process is shown in Figure 8B. To produce one functional unit, 1.76 kg $CO_{2,eq}$ was emitted. The electricity consumption of the freeze dryer (50%) and that of the fan (22%) had the largest contribution to the total impact. Approximately 14% of the impact was due to salt production, 5% to the production of nutrients and 4% to the CO_2 input. The production of electricity for the mixing process contributed 3% and production of electricity for the circulation pump and the centrifuge both contributed 1%. The other inputs had a negligible impact on climate change. In the 2012 pilot setup the drying step was clearly the least environmentally friendly process. It was therefore identified as needing optimization or replacement if possible.

Hypothetical: pilot plant 2013

Figure 8C shows the inputs of the pilot plant 2013 at the cradle-to-gate level. In this scenario, a total of 21.6 $MJ_{ex,CEENE}$ per MJ_{ex} DM was required from the natural environment. The biggest contribution to the total resource consumption was the electricity used to operate the fan (42%), followed by the electricity required to operate the freeze dryer (29%). A more efficient use of the freeze dryer resulted in a reduction of the impact of the drying step compared to pilot 2012. Also land use (8%), the input of nutrients (6%), the electricity use for mixing (5%) and the input of CO_2 (5%) had a relevant contribution to the total resource footprint. The other inputs had only a small contribution or are even negligible in this CEENE assessment. Nuclear resources were used the most (43%), mainly to supply electricity for the fan and the freeze dryer. In addition, 37% fossil resources were needed and 12% raw materials from the land occupation category and 6% from the water category. The fresh water requirements to grow the same amount of algae were thus projected to be much lower than in 2012, which can be explained by the recycling process.

The carbon footprint of the production of microalgae for this intermediate 2013 scenario is illustrated in Figure 8D. A total of 0.64 kg $CO_{2,eq}$ per MJ_{ex} DM was emitted. The electricity consumption of the fan (41%) and the freeze dryer (29%) had the largest contribution to the total impact on climate change. Approximately 11% of the impact originated from the CO_2 input, 9% from the production of nutrients and 5% from the production of electricity for mixing. The other inputs had a negligible environmental impact.



Figure 8 The resource footprint (A, C and E) and carbon footprint (B, D and F) of the production of dry algal biomass in pilot 2012 (240 m²) and the hypothetical pilot 2013 (1320 m²) and first production 2015 (2.5 ha) scenarios, respectively. The results are expressed per MJ exergy in the dry product.

Hypothetical: first production scenario (2015)

All inputs of the microalgae production system in 2015 are visualized in Figure 8E. In this scenario, a total of 2.46 $MJ_{ex,CEENE} MJ_{ex}^{-1}$ DM was projected to be extracted from the natural environment. The biggest contributors were the electricity production to operate the fan (25%), land use (22%), natural gas production for drying (21%) and the nutrient input (21%). Salt production (4%) and electricity use for mixing (3%) were also relevant, partly because the impact of the other inputs was reduced. In relative terms, a great deal of fossil raw materials were used (50%), mainly due to the use of natural gas. In addition, the impact of land use increased in relative importance (27%) because the larger PBRs, with more reactive surface, took up more land area. This scenario used Spain's electricity mix, which relied less on nuclear resources, and much less electricity was used than in previous scenarios. This reduced the impact of the nuclear category to only 9%. Water use added up to 8%.

In the 2015 scenario, 0.09 kg $CO_{2,eq}$ was emitted for the production of 1 MJ_{ex} dry algal biomass (Figure 8F). Again, the energy needed for drying was very important (32%), followed by the production of nutrients (30%) and electricity to operate the fan (28%). Other inputs had only a small to negligible effect on global warming.

Comparison between the three scenarios and a reference fish feed

Impact on resource consumption

Table 5 summarizes the distribution of the CEENE inputs on the eight impact categories for the three algae production scenarios in comparison to the CEENE requirements for the reference fish feed ($MJ_{ex,CEENE} MJ_{ex}^{-1}$ DM). In terms of total resource use, the 2013 scenario was 2.5 times better than pilot plant 2012. For the same amount of algae produced, the 2015 scenario used 23 times fewer natural resources than pilot plant 2012. This was mainly due to upscaling of the cultivation system, recycling of nutrient rich streams, use of flue gases, optimization of the PBR and the progress in reaching a higher photosynthetic yield together with better use of more energy efficient equipment. The biggest impact reduction can be found in the nuclear resources category because the electricity consumption declined substantially between the 2012 and 2015 scenarios. Furthermore, in the 2015 scenario, the algae facility used electricity from the Spanish grid, which had a smaller reliance on nuclear energy resources. These results clearly showed that even a small pilot plant could have a big impact on the environment. Nevertheless, remarkable progress appeared to be possible through upscaling, comprising a great potential for increasing the sustainability of microalgae production.

Table 5Resource Footprint ($MJ_{ex,CEENE} MJ_{ex}^{-1} DM$), Carbon Footprint (kg $CO_{2,eq} MJ_{ex}^{-1} DM$) and
Overall Exergy Breeding Factors (Dewulf et al., 2005) of the dry algal production in
the three scenarios and comparison with a reference fish feed.

	Pilot plant (2012)	Pilot plant (2013) ⁽¹⁾	First production Scale (2015) ⁽¹⁾	Reference fish feed ⁽²⁾
The CEENE method				
Renewable	0.58	0.18	0.11	0.20
Fossil	21.26	8.04	1.24	0.41
Nuclear	24.45	9.32	0.23	0.03
Metal ores	0.02	0.05	0.00	0.00
Minerals	0.17	0.01	0.01	0.00
Water	4.47	1.34	0.20	0.60
Land use	4.57	2.63	0.68	6.47
Atmosphere	0.00	0.00	0.00	0.00
Total	55.5	21.6	2.46	7.70
Carbon Footprint (CF)	1.76	0.64	0.09	0.05
Exergy Breeding Factor (BF _{ex})	1.03	1.09	1.28	1.00

⁽¹⁾ Hypothetical upscaling scenarios

⁽²⁾ (Huysveld et al., 2011, 2013)

Because the algal biomass can be used as fish feed, a comparison with the CEENE results of a traditionally produced reference fish feed were provided (Huysveld et al., 2011; Huysveld et al., 2013). The analysis of this fish feed (7.70 $MJ_{ex,CEENE} MJ_{ex}^{-1}$) was based on the same system boundaries and cut-off processes as used in this study, which makes it possible to objectively discuss the differences in sustainability between both types of fish feed. Table 5 shows that the microalgae production in 2012 had a greater environmental impact according to the CEENE method than a typical fish feed currently used for aquaculture purposes. Even in 2013, the impact on resource use was almost 3 times larger than conventional fish feed despite its 61% improvement towards pilot 2012. In the 2015 scenario, algae production used fewer raw materials during the life cycle and performed 68% better than the reference fish feed.

The distribution of the raw material consumption across the various categories differed between algae production and the reference fish feed production. Especially fossil and nuclear resource depletion occurred for algae production, followed by land occupation and uptake of water resources. In contrast, for production of the reference fish feed, the land use category clearly dominates. This could be explained by the algae's higher photosynthetic yield than the agricultural crops used to make the reference feed (Lardon et al., 2009). On the other hand, cultivating and processing algae was accompanied by higher energy use.

Impact on global warming

Table 5 provides an overview of the impact on climate change of all three algae production scenarios, as expressed in kg $CO_{2,eq}$ MJ_{ex}⁻¹ DM. Similar to the CEENE method, the results of the IPCC method showed that the pilot plant 2012 clearly had the biggest impact on the environment. The upscaled pilot facility 2013 showed great improvements with an impact reduction of 64%. The best scenario was the first production scale scenario 2015, which had an impact reduction of 95% within 3 years. Comparing the IPCC results of algae production with the results of the reference fish feed showed that even the best algae production scenario had still a way to go to reach a better or similar carbon footprint. This was in contrast to the CEENE results. The main reason was the consideration of land occupation in the CEENE method: traditional fish feed, which was largely based on terrestrial crops, scored worse than algae in this category.

It should be noted that the composition of the feed used for comparison in this study was made primarily from terrestrial biomass raw materials like soybeans, wheat and rice, while mainly the animal ingredients (such as poultry meal) had a large carbon footprint. The impact of commercial fish feed on the environment thus depends heavily on the type of ingredients and its composition. In practice, algae will probably replace a only fraction of currently used fish feed, such as fish oil and fish meal, because a 100% replacement may reduce fish growth performance because of a less efficient feed utilization (García-Ortega et al., 2016; Kissinger et al., 2016). From this perspective, microalgae are most likely to be used as an admixture in commercial fish feed. However, more tests are required with several types of algal species under different cultivation conditions and the effect on multiple fish species should be tested in order to exclude the possibility of a 100% replacement.

Exergy breeding factors

Table 5 gives an overview of the overall exergy breeding factors for the production of dry algal biomass at a cradle-to-gate boundary in the three scenarios. Because of the recycled water, nutrients, salt and vitamins and lower energy consumption in 2013 and 2015, the overall BF_{ex} became higher than in 2012. The algae concentration in 2015 was also higher (5g L⁻¹), contributing to a higher breeding factor (1.28). While producing dry algae, less exergy from non-renewable resources was used to reach the same exergy content of the reference fish feed now used in aquaculture. Dewulf et al. (2005) calculated a BF_{ex} of 12.57, 12.28 and 9.72 for rapeseed, soybeans and corn, respectively, which is in contrast to the algae production scenarios. According to the ecoinvent database, the dry matter content of the three above-mentioned terrestrial plants was 94%, 89% and 86%, respectively, related to the dry weight (DW)of the algal biomass (95%).

The difference in exergy breeding factors could be explained by the highly advanced cultivation of rapeseed, soybeans and corn, where further optimization had only a minor effect. In contrast, there are many known potential improvements to algae growth and development and some may be still unidentified.

4.4 Conclusions

Based on the results of the exergy analysis of all algae production scenarios, it is expected that the least exergy efficient processes (cultivation and drying) could be improved by a factor of 5 and 7, respectively, in only three years. Recycling of nutrients and savings on energy use were identified as important ways to increase the sustainability of algae production. Upscaling, reactor design improvements, enhancement of photosynthetic yield and a good choice of location also contributed to a lower resource and carbon footprint. Although additional efforts were required to improve the carbon footprint, algae production was projected to achieve a smaller resource footprint (2.46 $MJ_{ex,CEENE} MJ_{ex}^{-1} DM$) than traditional fish feed (7.70 $MJ_{ex,CEENE} MJ_{ex}^{-1} DM$) in the near future.

Chapter 5

Environmental sustainability analysis of a protein-rich livestock feed ingredient in the Netherlands: Microalgae production versus soybean import³

³ Redrafted from

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5.1 Introduction

5.1.1 Algae as a protein-rich alternative for soybean meal

According to the FAO agricultural outlook for 2015-2030, the consumption of animal products in the European Union (EU) will continue to increase, which is associated with a higher demand for vegetable protein feed sources. The supply of proteins in the EU for animal feed applications relies mainly on the import of soybean crops, which contain approximately 40% proteins and 18% lipids (fresh weight) (Dutch Soy Coalition, 2008). Soybean meal contributes to 73% of the total protein-rich meals commonly used in the EU feed industry (Figure 9).



^(b) From imported soybeans and soybean meal

^(c) Includes meal from sunflowers, copra, cottonseed, peanuts, palm kemel, and other protein meal sources

Soybeans are annual crops that grow best in (sub-)tropical climate regions with many warm but rainy months combined with high temperatures and a long day. Due to the lack of these climatic conditions, many European countries are forced to import this essential protein source for feeding their livestock. Countries such as Brazil, USA and Argentina,

Figure 9 Different protein sources used in the EU livestock and feed industries (American Soybean Association, 2008)

which have such local weather conditions, are the main exporters of soybeans and soybean meal (Dutch Soy Coalition, 2010). Based on data from 2006 to 2010, a yearly average of 13.9 million tonnes of soybeans and 22.1 million tonnes of soybean meal are imported to the EU. The soybeans originate mainly from Brazil (57%), followed by the United States (22%) (Eurostat, 2012). The Netherlands accounts for 26% of the EU soy import and plays a crucial role in the soybean market. The largest soybean traders in the world, Cargill and ADM, are located in the ports of Rotterdam and Amsterdam. Approximately 75% of the soybeans that enter these plants are crushed into soybean oil (20%) and meal (80%); the latter is commonly used in animal feed for dairy cattle, chickens, pigs and calves. The other 25% is directly exported to other European countries (Dutch Soy Coalition, 2010).

Today, questions have arisen about the environmental sustainability of the whole process. The nutrient cycle can be disturbed when soybean cultivation and livestock production are geographically separated. The manure of the animals that are fed with soybean products is not available as fertilizer for the land that is used in the feed production. The soybean importing regions must face waste problems, and the exporting regions experience problems with soil depletion (Aprodev EU CAP, 2011). The high demand for soy affects the exporting countries because they want to pursue higher yields, which results in soil degradation and erosion (Dutch Soy Coalition, 2008); additionally, when there is increasing land use, it is a threat to biodiversity in countries such as Brazil, where deforestation of the Amazon rainforest and the Cerrado biome (tropical forest savanna) is occurring (Fearnside, 2001). Approximately 2.31 million hectares of forest per year disappear, which is approximately 70% of the land area of the Netherlands. Additionally, transporting tonnes of soybeans over a long distance requires a large amount of infrastructure and results in ecological problems due to non-renewable fossil fuel use and the release of emissions (Prudêncio da Silva et al., 2010). In addition, from an economical point of view, it is interesting to change from soybeans to other types of protein-rich feedstock because soy prices are maintained at generally high levels due to the market power of (among others) China, the world's largest soybean importer (Song et al., 2009).

To decrease the soybean demand, European countries must be more self-sufficient in producing protein-rich biomass. Taking into account the low availability of fertile land in

Europe, an increasing interest in the fast growing protein rich algae could be observed as they can be grown on marginal land, potentially in combination with waste treatment (section 1.2). The choice of cultivation system and processing steps often depends on the available capital and the final biomass in the application. Because of the emerging concern over fuel source depletion, efforts are currently being made to produce algae for fuel applications such as biodiesel, bio(syn)gas, bioethanol and biohydrogen. However, according to (Passell et al., 2013), the production of these low value algae-based products involves worse performance than the existing (petroleum based) alternatives because the energy embodied in the algal fuel is lower than the energy required to produce it. Consequently, the focus has shifted toward higher value applications, such as animal feed.

According to a study of Pulz and Gross (2004), it has been proven that some algae species such as *Chlorella, Scenedesmus* and *Spirulina* have beneficial effects on the health of animals: improvement in their immune response system and fertility, a healthier coat and better weight control. For this reason, protein-rich algal meal is a promising alternative to soybean meal, which is now the main protein source in the animal feed sector (Lum et al., 2013). Of course, to substitute soybean meal, algae should meet some other requirements: high protein levels, good amino acid quality, digestibility and low price (De Visser, 2013). Several algae species have a dry matter crude protein content of approximately 50%, which is higher than soybeans (44%), and the amino acid profile appears to be well balanced and is (similar to the digestibility) comparable with soybeans (Polprasert, 2007; Becker, 1994).

5.1.2 Environmental resource footprint of protein-rich meal

In this early phase of development, it is essential to consider the sustainability of the algae production, to have minimal environmental and social impact combined with maximal economic value. Probably the best way to reach a low environmental footprint is to switch from a linear economy to an integrated biorefinery concept in which algae are produced close to an industrial facility, which delivers its own products, and where waste heat, waste water and flue gases are used to stimulate the algal growth (Aitken and Antizar-Ladislao, 2012). This concept is beneficial for the joining industries: on the one hand, the required products are made, and on the other hand, sustainability-related issues such as land

occupation, fossil fuel use and greenhouse gas emissions can be mitigated. In addition, when algae cultivation and processing is positioned near its sale market, e.g., livestock production, it creates a win-win situation for all of the associates.

To steer this development in a sustainable way, this study determines the natural resource footprint of protein-rich algal meal for livestock feed applications in the Netherlands. Microalgae are cultivated at a pilot scale (500 m² open ponds) and are integrated in a biorefinery (anaerobic digester, combined heat and power (CHP), condensers), making use of waste heat and flue gases. The final products are electricity, digestate, heat available for a nearby bio-ethanol facility and algae oil and meal. The sustainability of this rather new biomass source for animal feed is compared with the more traditional route of soybean crop production. The soybeans are cultivated, dried and crushed in Brazil, and the soybean meal, which is commonly used as a protein-rich animal feed ingredient, is transported to the Netherlands. First, an exergy analysis at the process and gate-to-gate level is performed on the integrated algal biorefinery, to visualize the process (in)efficiencies of this rather new technology. Furthermore, an attributional LCA at a cradle-to-gate level is performed to determine the environmental sustainability of a product such as algal meal. In this study, the resource footprint of a basket of products delivered by the linear (soybean based) economy and (algae based) biorefinery is determined. Additionally, a sensitivity analysis is conducted to examine possible avenues for improvement in the biomass production technology.

5.2 Materials and methods

The focus of this study is to assess the process inefficiencies and environmental sustainability of algae cultivation at a pilot scale in a biorefinery, producing (among other things) algal meal as a protein-rich feed ingredient for livestock. Therefore, an EA at the process and gate-to-gate level is conducted for the biorefinery. Furthermore, the environmental resource footprint is calculated on a life cycle basis. To interpret the LCA results of the algae production scenario thoroughly, a comparison with a similar product is

made: soybean meal. Algae production took place in the Netherlands, and the meal was fed to local cattle, while soybeans were cultivated and processed into meal in Brazil and transported to Rotterdam, one of largest harbors of the Netherlands.

5.2.1 Process description and inventory

The production of two protein-rich feed ingredients, algal meal and soybean meal, is described in the sections below. The product systems deliver the same basket of products (functionalities), on the one hand produced in a biorefinery and on the other hand in a linear economy.

Protein source (alternative 1): Algal meal

Figure 10 represents a schematic overview of the full process chain (foreground system) of the integrated algal biorefinery. In the Netherlands (Lelystad), a mixture of photoautotrophic microalgae *Scenedesmus* and *Chlorella* are cultivated in two separated ponds: each pond had a surface area of 250 m² and a depth of 0.6 meter. These ponds are located at the ACRRES (Application Centre of Renewable Resources) facility, which is part of Wageningen University and Research Centre (WUR). In cooperation with the company Eneco and the joint venture Algae Food and Fuel, the algae production plant was constructed in 2012. On the ACRRES site, two digester units processed cattle manure, silage maize, maize straw and feed residues (silage maize and grass) to produce an energy-rich biogas (Figure 10). The biogas is recovered and burned in a CHP installation, generating green electricity that is delivered to the national grid. During the combustion, warm CO_2 rich flue gas is released. The carbon dioxide of the flue gas is used in the algae cultivation systems as a carbon source. To avoid overheating of the CHP, water is used as a medium to transport the heat to the digesters, which are operative at a mesophilic temperature.

The remaining product of the digester unit, digestate, is used as a fertilizer on nearby fields to decrease the use of mineral fertilizers. In the near future, the liquid fraction of the digestate could be used to feed the algae but, inducible from a small-scale experiment at the site, further research is necessary to decolor/filter the digestate because the turbidity distorts the light penetration and the possible presence of heavy metals could negatively affect the algal growth.



Figure 10 Process flow scheme of the integrated algal biorefinery in Lelystad (foreground system, gate-to-gate boundary indicated with red line); the end products (digestate, electricity, heat, algal oil and algal meal) are marked with a black frame. Several units can be distinguished: digesters unit, Combined Heat and Power (CHP), condensers, inoculum production system, algae cultivation ponds, oalescers and centrifuges, dryer and oil extraction step. The last two processes are based on literature data (dotted line), while the upstream processes are based on data collected directly on-site (full line).

The flue gases from the CHP (400°C) are cooled down in two condensers that are placed in sequence. In the first condenser, the gas is cooled to 120°C, and in the meantime, heat (hot water) is provided. This heat is one of the end products of the biorefinery, which is used in a nearby bio ethanol installation. In the second condenser, the temperature of the gas drops to 50°C. Again, hot water is produced, which is used for heating the algae ponds. Coupling the digester and algae units had some advantages: waste streams such as flue gases could be used, providing heat and CO_2 . Only a part of the flue gases entered the algal system, and the gases that are in excess are released to the atmosphere through a pressure relief valve.

The inoculum is prepared in a sequence batch system that consists of three separate successive tanks with a volume of 1 m³ (T6), 20 m³ (T3) and 60 m³ (T4), respectively. Necessary nutrients (mainly nitrogen and phosphorous) are added at the start of the batch, and artificial lighting is used to stimulate the biomass growth. Air sparging of a part of the available flue gases occurred only in T3 and T4, and the tanks are heated through the available hot water provided by the second condenser. Only in the smallest tank (T6) mixing occurred. On average, the inoculum procedure took place four times a year (12-day periods) to offer a start-up of the ponds when necessary. More information can be found in Appendix A, Table A.1.

One of the algae ponds is located in a greenhouse, and the other is located outside (for experimental research). This arrangement affected some of the parameters, such as the temperature of the ponds, rain input in the outdoor pond and extra infrastructure (glass of the greenhouse) for the indoor pond. Apart from these small differences, both ponds operated very similarly: small amounts of KNO₃, KH₂PO₄ and micronutrients are added each day (Appendix A, Figure A.1), flue gases (source of CO₂) are blown into the ponds with a Van Dijk ventilator (1.5 kW) and BOSA ventilators (SD600,2.7 kW, SD400, 1.8 kW) and mixing occurred (Flight Mixer 0.9 kW). The outdoor pond had a temperature of approximately 5°C, and the indoor pond 10°C (yearly averages). Extra heat is required to maintain these temperatures, especially in the colder seasons; a combination of heat available in the flue gases and hot water from the second condenser that flowed through the pipes under the ponds is used. Every hour, approximately 6 m³ (0.27% DM) per pond is

withdrawn and pumped to a coalescer (each pond had one coalescer, Appendix A, Figure A.2), where the algae could sink due to specific flow patterns that induced auto flocculation, i.e., no energy is consumed at this stage (except for pumping).

In total, 2 m³ (0.29% DM) is retained in the coalescers on a daily basis; approximately 0.49 kg DW algal biomass is harvested (annual average productivity of 1 g m⁻² day⁻¹). The part of the biomass that did not sink is recycled back to the respective algae pond. The harvested fractions are then dewatered using a SMB Apeldoorn centrifuge (one device per pond is available, operating 1.5 hours day⁻¹), where approximately 90% of the algae are harvested. In total, an average of 3241 kg DW ha⁻¹ year⁻¹ is harvested. The concentrate (10.2% DM) is sent to the drying equipment, and the centrate is discharged into the sewer. Both of the coalescers, similar to the centrifuges, are located in the greenhouse.

For the digestion of biomass, the burning of biogas in the CHP, cooling the flue gases in the condensers, algae production and downstream processing, data about the material (infrastructure included) and energy flows are gathered directly on-site and obtained through further communication with experts in the field of algae production and LCA. Transport of the resources (e.g., cattle manure, maize products and feed residues for the digesters) and final products (such as digestate) could be neglected because the production units (livestock, agricultural products, algae) are situated at the same location. For the drying and extraction step, data are used from the literature and/or databases because these processes are not operational yet. According to a study of (Aitken and Antizar-Ladislao, 2012), it is stated that it is more efficient to dry the algae before extracting the oil. A natural gas-fired dryer is used to dehydrate the algae stream to 89% DM, which is a good percentage for a stable conservation. Data about the energy consumption (3,556 kJ kg⁻¹ evaporated water) of the drying method is found in the study of (Sander and Murthy, 2010). Because oil extraction/meal production from algae is very similar to that of soybeans (Kiron et al., 2012; Sazdanoff, 2006), data about soybean processing into oil and meal is used from the inventory database ecoinvent version 2.2 (Frischknecht and Rebitzer, 2005). More detailed information about the LCI data of the integrated algal biorefinery is available in Table A.2 of Appendix A. It is estimated that at least 80% of all of the data are collected directly on-site, and other essential data are computed through mass and energy balancing or are found in the literature (less than 5% of the data).

This pilot setup leads to a yearly production of 2453 kg meal (DW) and 591 kg oil (DW) per hectare. The extracted oil can be used (perhaps after refining) in pharmaceuticals, chemicals and human food or can be further processed into useful energy sources. The algal meal can be used as an important protein source for livestock feed.

Protein source (alternative 2): Soybean meal

In the study of (Prudêncio da Silva et al., 2010), the environmental sustainability of soybean production in Brazil and transportation to the Netherlands is assessed. Because soybean production and its supply chain are highly dependent on land, pesticides, fertilizers, machinery, transport, fuel and electricity, these inputs are accounted for. The two primary supply regions of soybean crop production are identified: the Center West (CW) and Southern (SO) regions of Brazil. Each region comprises different cultivation strategies that are related to the used fertilizer type and tillage system. A zero-tillage system is used for 80% of the crop production in both regions, and only in the SO region, pig slurry is used, next to chemical fertilizers (3.6% of the total soybean crop production area received manure). Figure 11 illustrates the relative contribution of each soybean production scenario, and the soybean general composition is given.

Nitrogen and phosphorus emissions from crop production are calculated for each scenario, and the differences in weather conditions, mineralization of crop residues, leaching, runoff, volatilization and soil losses are accounted for. The CW region suffered from land transformation: it is estimated that 1% of the soybean production area is transformed from rainforest and 3.4% from Cerrado. In the SO region, rainforest and Cerrado land types do not exist, and it is assumed that no transformation of other land types took place. As a yearly average, the harvested amount of soybeans at 18% water content amounted to 2612 kg DW ha⁻¹.



Figure 11 The two main soybean supply regions of Brazil: Center West (CW) and Southern (SO). Each region comprises different cultivation strategies.

After cultivation, the beans are processed. Data about pre-cleaning, cleaning and storage of the beans are gathered from (Marques, 2006). Data about the grain drying process was available in the LCI database ecoinvent (version 2.2), but the process is optimized according to the Brazilian situation, in which wood chips are used to dry the soybeans instead of fuel oil (Marques, 2006). Transportation from the different cultivation locations to the oil mill is accounted for. According to their estimates, approximately 70% of the soybeans delivered at the oil production site are from the CW, and 30% are from the South (Figure 11).

The crushing of dried soybeans with 11% water content is based on the oil extraction process that is available in ecoinvent version 2.2 (Weidema and Hischier, 2010), but again this process is adapted to the Brazilian reality (gathered by personal communication with Prudêncio da Silva). Approximately 1980 kg of soybean meal (DW) and 475 kg of soy oil (DW) per hectare could be produced (yearly average). Finally, soybean meal is transported to the seaport in Rotterdam. Therefore, the main road, rail and water ways are identified for both regions to the Netherlands and amount to 2048.63 tkm, 378.39 tkm and 9980 tkm, respectively (personal communication with Prudêncio da Silva). For a complete data inventory, we refer to the study of Prudêncio da Silva et al. (2010).

5.2.2 Exergy analysis: process and gate-to-gate level

The physical, chemical, electrical and radiation exergy is calculated according to the equations described in section 4.2.2 (Taelman et al., 2013). The group contribution method, Gibbs calculations and β low heating value method are used to obtain the exergy content of the different flows (De Vries, 1999; Kotas, 1995; Shieh and Fan, 1982). For example, the exergy content of the amino acids, fatty acids, sugars and cellulose components of the algae and soybeans are calculated according to the group contribution method (Becker, 2007; Jena et al., 2012; Taelman et al., 2014). A composition analysis of the algae cultivated in Lelystad is performed by NutriControl and resulted in 10% lipids, 20% carbohydrates, 52% proteins and 18% ash. This composition is used to calculate the average exergy content of the algal meal (18.57 MJ_{ex} kg meal⁻¹) and algal oil (39.48 MJ_{ex} kg oil⁻¹). The study of Chowdhury et al., 2007 revealed the fatty acid composition of soybean oil, and its exergy content is calculated as 39.20 MJ_{ex} kg oil⁻¹. Soybean meal had an exergy content of 20.64 MJ_{ex} kg meal⁻¹ (Huysveld et al., 2013, personal communication). Additional information about the detailed calculation of the exergy content of algal and soy oil and meal can be found in Tables A.3 to A.5 of the Appendix A.

An exergy balance is made for the foreground processes of the integrated algal biorefinery: digesting, CHP process, condensing, inoculum production, algae cultivation, dewatering (coalescers and centrifuges), drying and crushing. This process chain delivers algal oil and meal, electricity, useful heat and digestate (digestate is used as fertilizer). At the process and gate-to-gate level, the exergy efficiency Ex_{eff} is calculated according to equation (12); Ex_{out} represents the exergy contained in the final products, and Ex_{in} represents the exergy contained in the final products at the respective levels. Possible improvements are suggested for the most inefficient processes.

$$Ex_{eff} = \frac{Ex_{out}}{Ex_{in}}$$
(12)

5.2.3 LCA: cradle-to-gate level

The framework of the International Standards Organization (ISO) 14040 and 14044 is followed (ISO, 2006a,b) to assess the environmental sustainability of the algal and soybean product systems (explained in section 2.1). An environmental systems analysis (ESA) using attributional LCA methodology is conducted in this study (Finnveden et al., 2009). System expansion based on functionalities is used as an alternative technique to address the allocation (which should be avoided according to the ISO standards). Therefore, when no product-specific information is necessary, it shifts the LCA to a broader (black box) study, where the focus is placed on a basket of products using system expansion is often associated with CLCA, we consider it to be an attributional approach because no consequences are addressed (Zamagni et al., 2012).

Goal and scope

A cradle-to-gate (exergetic) resource footprint of both product systems, i.e., the soybeanbased linear economy and the integrated algal biorefinery, is examined. System expansion is used at a black box level. This aspect changed the scope from an LCA study to an ESA using an LCA approach, which implies several functional units (FU). Comparisons between the different product systems are made on the basis of the same functions and are quantified by the functional units. In this study, the basket of products to which the environmental impact is assigned is 1 MJ_{ex} (algal/soy) meal, 0.51 MJ_{ex} (algal/soy) oil, 986.62 MJ_{ex} electricity, 139.15 MJ_{ex} heat and 1015.76 MJ_{ex} digestate. A protein-rich meal is chosen to be the reference product (Figure 11). However, a 6th function can be detected from the algal biorefinery, namely the treatment of waste streams (e.g., cattle manure), i.e. getting rid of the excess manure. However, in this case, waste becomes a valuable resource for the digester unit and useful products (digestate and biogas) are produced, which are accounted for in the basket of products. This is a clear example of a technology with multiple functions, however, in LCA this is dealt with separately by focusing on the amount of waste treated or on the products produced. In this analysis, we focus on the production side of the technology and therefore only consider the useful outputs, energy and fertilizer (Prosuite, 2013).

In the integrated algal biorefinery, several products are produced, including algal meal as a protein-rich cattle feed ingredient (Figure 10). To make a fair comparison with the soybean product system, it is necessary to deliver the same functionalities. Therefore, the resource footprint of producing heat, electricity and digestate (similar to the systems of the algal biorefinery) is added to the footprint of the soybean meal (and oil) production, which is a linear economy that produces the same functionalities as the biorefinery (Figure 12).



Figure 12 Comparison between the integrated algal biorefinery and the soybean based linear economy where the same (amounts of) functionalities were produced. System expansion was used to avoid allocation.

Furthermore, a sensitivity analysis is conducted to examine possible improvements in the algae production. The sensitivity of the resource footprint results of the integrated algal biorefinery toward several parameters (the resources used for electricity production in the Netherlands, the working hours of the blowers and mixing devices, the types of the blowers, the harvested biomass fraction and the algal productivity) is evaluated. Table A.6 in Appendix A shows a brief overview of the selected parameters for the sensitivity test.

Life cycle inventory and impact assessment (LCI(A))

During the inventory analysis, all of the resource inputs and emissions are quantified and related to the basket of products. A description of the data inventory of the foreground processes can be found in section 5.2.1. Both of the product systems have similar system boundaries. The background data of both alternatives is found in the database ecoinvent version 2.2 (Frischknecht and Rebitzer, 2005). The resource-based CEENE method (2013) is used to calculate the environmental resource footprint (8 categories) of the five different
functionalities (Dewulf et al., 2007a; Alvarenga et al., 2013). This LCIA method quantifies the exergy deprived from nature to produce soybean/algal meal. As explained in the paper of (Alvarenga et al., 2013), exergy-based spatial explicit characterization factors (CF) for land occupation are calculated. To assess the resource footprint of cultivating soybeans in Brazil, a CF of 38.8 MJ_{ex} m⁻² year⁻¹ is used, and for algae production in The Netherlands, a CF of 25.3 MJ_{ex} m⁻² year⁻¹ is obtained (supporting information (SI) of Alvarenga et al., 2013).

5.3 Results and discussion

The process (in)efficiencies of the integrated algal biorefinery are exposed during the EA, and the results can be found in section 5.3.1. The discussion of the results of the sustainability analysis at the cradle-to-gate level is shown in section 5.3.2.

5.3.1 Exergy analysis of the integrated algal biorefinery

This study revealed the process inefficiencies of algae cultivation in open ponds while making use of waste streams in a biorefinery, as well as algae drying and meal production. Two different harvesting and dewatering approaches are applied at a pilot scale, and the results for both scenarios are explained below. The physical, chemical, electrical and radiation exergy of the input and output flows are calculated. The exergy efficiency for every process of the biorefinery is obtained as well as the exergy efficiency at the gate-to-gate level (foreground system).

Scenario 1: harvesting and dewatering with two coalescers and two centrifuges

As seen from Table 6, the most exergetically inefficient processes are anaerobic digestion (66.47%), condensation (56.53% and 63.81%), inoculum production (54.98%) and drying (44.01%). Per day, $3.69 \cdot 10^4$ MJ_{ex} from cattle manure, maize products and feed residues is digested, and digestate ($6.34 \cdot 10^3$ MJ_{ex}) and biogas ($1.97 \cdot 10^4$ MJ_{ex}) are produced, which leads to the highest exergy loss of all of the processes ($1.31 \cdot 10^4$ MJ_{ex}). The exergy content of all of the electricity that is used for processes such as mixing and pumping, which relate to the digester unit, and the exergy content of the heat input to maintain a mesospheric

temperature range are lost. Furthermore, the conversion from solid/liquid biomass feedstock to a gaseous phase (biogas) affected the performance of the digesting process in such a way that additional entropy is produced, i.e., the exergy content of the biogas that is produced is lower than the exergy content of the digestible organic matter.

Process	Exergy content	Exergy content	Exergy
	inputs (KJ _{ex} FU ⁻¹)	outputs (KJ _{ex} FU ⁻¹)	efficiency (%)
Anaerobic digestion	6.20 x 10 ⁶	4.12 x 10 ⁶	66.47
CHP process	3.12 x 10 ⁶	$2.42 \ge 10^6$	77.66
Condensation (part 1)	3.98 x 10 ⁵	2.25 x 10 ⁵	56.53
Condensation (part 2)	1.09 x 10 ⁵	6.98 x 10 ⁴	63.81
Inoculum production (T6,T3,T4)	$1.28 \ge 10^4$	7.04×10^3	54.98
Algae cultivation, outdoor pond (T2)	1.66 x 10 ⁶	1.22 x 10 ⁶	73.21
Algae cultivation, indoor pond (T1)	1.65 x 10 ⁶	1.21 x 10 ⁶	70.80
First dewatering (coalescer) T2	1.22 x 10 ⁶	1.21 x 10 ⁶	99.46
First dewatering (coalescer) T1	1.22 x 10 ⁶	1.21 x 10 ⁶	99.46
Second dewatering (centrifuge) T2	1.06 x 10 ⁴	8.93 x 10 ³	84.22
Second dewatering (centrifuge) T1	1.10 x 10 ⁴	8.90 x 10 ³	80.63
Drying process	3.38 x 10 ³	1.49 x 10 ³	44.01
Crushing process	1.72 x 10 ³	1.50 x 10 ³	87.43

 Table 6
 Exergy efficiencies of the foreground processes of the algae based biorefinery.

The function of the condensers is cooling down the hot flue gases (400°C). These flue gases have a physical and chemical exergy value that amounts to 2.48 x 10^3 MJ_{ex} day⁻¹ (first condenser). In the meantime, water could be heated and used in another unit. The heat that became available (8.68 x 10^2 MJ_{ex} day⁻¹) together with the remaining flue gases resulted in an exergy output of 1.42 x 10^3 MJ_{ex} day⁻¹ for the first condenser. Heat is lost to the environment within both condensing processes, and the difference in the exergy efficiency can be explained by the fact that lower heat losses occurred with lower flue gas temperatures.

Electricity (10.11 kWh per day) for artificial lighting, cooling, mixing and blowing of $CO_{2^{-}}$ enriched air is used to achieve optimal growing conditions in the inoculum tanks. The electricity consumption had a contribution of 45% of the total incoming exergy. Predominantly, the blower and led lights are the main consumers. As an alternative,

mirrors to capture more daylight and less energy-intensive blowing equipment (<1.8 kW) could be used, with fewer operating hours for the blowing equipment whenever possible.

The results from the EA revealed the most exergetic inefficient process: drying. The algae stream of 10.2% DM had to be dried to 89% DM, which required 3.9 kWh day⁻¹ of natural gas to remove 3.95 kg of water each day. To reduce the energy-intensity of this step, solar and/or wind drying as a first drying step could be used, but this technology is very dependent on the local climate (Sander and Murthy 2010). The latest research on direct oil extraction from wet algae biomass (78%-93% moisture) and the promising results showed important routes to make the overall process more sustainable (Kanda and Li, 2011).

Scenario 2: harvesting and dewatering with four centrifuges

In the processes that are related to electricity production (CHP) and algae cultivation (sunlight is accounted for), dewatering and crushing had observed exergy efficiencies that were higher than 70%. These steps (except for the first dewatering) can be further optimized, but optimization is not the priority from an environmental (resource) point of view. Nevertheless, the experts of the algae pilot attempted to improve the harvested biomass yield by removing the coalescers and by placing two additional centrifuges. At that moment, four centrifuges operating at 16 hours day⁻¹ had to process a low concentrated algae stream (0.3% DM), and only approximately 15% could be harvested (648 g day⁻¹). The remaining algae that is present in the centrate is recycled back to the ponds.

In scenario 1, the average exergy efficiency of the cultivation processes (pond T2 and T1) and the dewatering processes (coalescers and centrifuges) for both ponds is 72.00% and 90.94%, respectively. In scenario 2 (four centrifuges), only 2 m³ hour⁻¹ (four pumps operating each at 16 hours day⁻¹ at 0.75 m³ hour⁻¹) is tapped from the ponds. This resulted in a low exergetic efficiency for algae cultivation of 15.69% (1.75 x 10^6 KJ_{ex} FU⁻¹ inputs versus 2.74 x 10^5 KJ_{ex} FU⁻¹ outputs). The exergy efficiency of the new dewatering setup (four centrifuges) amounts to 80.20%. The processing of more biomass per day also had an effect on the drying and crushing exergy efficiencies: 47.24% and 90.74%, respectively. Thus, the cultivation and dewatering processes of the new setup were exergetically less efficient than in the original setup, but the drying and crushing are improved. As mentioned before, only a relatively small part of the produced algal biomass is harvested with the centrifuge.

During the test period with the four centrifuges, a shift to a smaller algae species was observed to decrease the harvestability with the centrifuge. Thus, we could not yet make a conclusion about which setup is the most environmentally friendly.

Comparison between the two scenarios

At a gate-to-gate level, the exergy efficiency is 30.14% for the original setup. At this level, the largest contributors to the overall exergy input are biomass sources such as cattle manure, feed residues, and maize straw (together, 83.12%). For the second scenario, the overall exergy efficiency is 29.81%; despite more algal oil and meal production per day, even more energy is consumed (in relative terms). As a result, no significant improvement could be detected compared with the first setup. More details related to the contribution of all of the inputs and outputs of the foreground system can be found in Table A.7 of the Appendix A.

5.3.2 LCA of protein-rich meal production from algae versus soybeans

The EA at the gate-to-gate level revealed no significant improvement in the second scenario with four centrifuges compared with the original setup (coalescers and centrifuges). Therefore, a cradle-to-gate ESA (LCA) study is conducted for the original setup, as described in section 5.2.1. The system expansion approach is used to address the allocation. The results are expressed for five different products (functional units): 1 MJ_{ex} (algal/soy) meal, 0.51 MJ_{ex} (algal/soy) oil, 986.62 MJ_{ex} electricity, 139.15 MJ_{ex} heat and 1015.76 MJ_{ex} digestate. The resource footprint of the integrated algal biorefinery is compared with the footprint of importing soybean meal to the Netherlands (section 5.2.1). A sensitivity analysis was conducted, which revealed the possibilities for reducing the resource footprint in the near future .

Environmental resource footprint of the integrated algal biorefinery

Table 7 shows the resource footprint of the integrated algal biorefinery. As seen from this table, the total resource footprint of the biorefinery, as explained in section 5.2.1, is 3033.72 MJ_{ex} , which is derived from nature to produce the basket of products. A hotspot analysis identified the major contributors to the respective total resource footprint and the different resource (impact) categories. The largest contributor (73%) to the total

footprint is the digesting step in which biogas and digestate is produced. The biomass inputs to the digesters (especially silage maize) had a large resource footprint due to the large daily request for digestible material (Table A.2, Appendix A). In this study, cattle manure and feed residues are assumed to be waste products that could be valorized, i.e., no environmental impact is assigned to it, which follows the zero burden assumption (Ekvall et al., 2007). Furthermore, in the dosage system, mixing and pumping required a large amount of electricity (339 kWh day⁻¹).

The cultivation steps in the outdoor and indoor ponds are the second and third contributors. The total resource footprint of both ponds is very similar, but some variations between the different resource categories can be noticed. Because the pond that is located inside the greenhouse experienced a higher ambient temperature of approximately five degrees Celsius, some of the parameters are not the same: (1) the pump that is responsible for providing heat (hot water) to the ponds had lower operating hours for pond T1, (2) the electricity consumption of the flue gas blower is slightly lower for pond T1 because less heat is necessary, (3) the nutrient measurement (yearly averages) are not the same between the two ponds, which results in the net input of nitrogen and phosphorous being different, and (4) extra infrastructure for the greenhouse construction had to be accounted for. Especially the electricity consumption for the different blowers and mixing devices had a high impact on the resource use; it contributed 89% of the resource footprint for the algae cultivation.

The CHP process had a relative contribution of 6.14% to the total resource footprint of the biorefinery. The CHP itself did not consume any energy, but the electricity consumption of the corresponding emergency gas cooling system and pumps is responsible for a contribution of 95% of the footprint of $1.86 \times 10^2 \text{ MJ}_{ex}$ (5% for infrastructure). The other processes (e.g., dewatering, condensing) contributed less than 1% to the total environmental resource footprint. Surprisingly, the drying step had the second lowest contribution, which is in contrast to the EA results, in which drying performed the worst.

The most exploited natural resources at the cradle are land resources (47.16%) and fossil fuels (43.44%). Both of these resources are most often used within the digesting process;

the cultivation of agricultural crops requires a high land use, and the electricity production in the Netherlands is highly dependent on natural gas and coal (fossils). Mainly, electricity is consumed for mixing, pumping and proportioning (dosage system). To produce the basket of products, $1.34 \times 10^2 \text{ MJ}_{ex}$ nuclear resources (4.43%) and 5.38 x 10¹ MJ_{ex} abiotic renewables (1.77%) are extracted, which are also mainly for the production of electricity. Approximately 4% of the total resource footprint is caused by the use of fresh water, especially during digestion and algae cultivation. More detailed information can be found in Table A.8 of Appendix A.

Table 7Resource footprint of the integrated algal biorefinery in the Netherlands using CEENE as LCIA method for the production of several
functionalities: 1 MJ_{ex} algal meal, 0.51 MJ_{ex} algal oil, 986.62 MJ_{ex} electricity, 139.15 MJ_{ex} heat and 1015.76 MJ_{ex} digestate. The relative
contributions to the total resource footprint for the different processes and impact assessment categories were calculated.

	Abiotic	Fossil	Nuclear	Metal	Minerals	Water	Land use	Atmos-	TOTAL	Relative
MJ _{ex} /functionalities	Renewables	fuels	resources	ores				pheric		contribu-
								resources		tion (%)
Digestion	2.64E+01	6.86E+02	6.66E+01	5.51E-01	9.04E-01	4.64E+01	1.41E+03	0.00E+00	2.21E+03	72.74
CHP process	6.35E+00	1.48E+02	1.53E+01	7.40E-02	3.68E-02	1.17E+01	4.23E+00	0.00E+00	1.86E+02	6.14
Condensation	3.01E+00	7.08E+01	7.30E+00	1.57E-02	1.51E-02	5.58E+00	1.99E+00	0.00E+00	8.87E+01	2.93
Inoculum production	1.76E+00	2.67E+01	5.51E+00	3.22E-02	2.50E-02	8.62E+00	9.40E-01	0.00E+00	4.36E+01	1.44
Algae cultivation T2	7.12E+00	1.68E+02	1.74E+01	8.16E-02	5.12E-02	2.30E+01	7.60E+00	0.00E+00	2.24E+02	7.37
Algae cultivation T1	7.10E+00	1.68E+02	1.73E+01	8.18E-02	5.33E-02	2.30E+01	7.59E+00	0.00E+00	2.23E+02	7.35
Dewatering T2	1.00E+00	2.35E+01	2.42E+00	7.18E-03	5.79E-03	1.85E+00	6.65E-01	0.00E+00	2.94E+01	0.97
Dewatering T1	1.00E+00	2.35E+01	2.42E+00	7.18E-03	5.79E-03	1.85E+00	6.65E-01	0.00E+00	2.94E+01	0.97
Drying	7.69E-03	2.79E+00	1.39E-02	1.95E-04	2.92E-04	5.54E-03	2.54E-03	0.00E+00	2.82E+00	0.09
Crushing	2.06E-03	1.32E-01	5.07E-03	1.29E-05	2.35E-05	4.34E-03	2.10E-03	0.00E+00	1.46E-01	0.00
TOTAL	5.38E+01	1.32E+03	1.34E+02	8.51E-01	1.10E+00	1.22E+02	1.43E+03	0.00E+00	3.03E+03	
Relative contribution (%)	1.77	43.44	4.43	0.03	0.04	4.02	47.16	0.00		

Comparison with the environmental resource footprint of the linear economy with soybean production

The functionalities produced in the biorefinery, as described in the previous sections, could be produced under different circumstances/production routes. The heat, electricity and digestate are produced anyhow, and can be seen as a first part of a linear economy, which takes place at the same location (ACRRES site in Lelystad). Therefore, the resource footprint for this part is the same as for the linear economy and the biorefinery and a further discussion is therefore not relevant. The second part of the linear economy consists of the Brazilian soybean cultivation and oil and meal production, of which the latter is until now the main protein-rich ingredient in livestock feed. In this study, it is assumed that soy-based or algae-based oil and meal have the same functionalities.

Table 8 gives a brief overview of the resource footprint of soybean meal and microalgal meal production, of which the latter is produced with waste streams from the biorefinery. Under the current situation, a difference in the resource footprint of a factor of 10² can be detected. Soybean cultivation requires a large amount of land resources, which entail a relative contribution of 88.85% to the total resource footprint of cultivating soybeans for soybean meal (1 MJ_{ex}). The second most used raw materials are fossil resouces, which is mainly due to the fuel use of the agricultural machinery (1.24 MJ kg⁻¹ soybeans) that is operational during biomass cultivation and harvesting and because of the energy used for the production of chemical fertilizers (e.g., triple superphosphate). In contrast, during algae cultivation, mainly electricity is used for blowing and mixing (28.96 MJ kg⁻¹ DW algae). The supply chain of producing electricity includes the use of several resources such as fossil resources, land and water, nuclear and abiotic resources and metals. The production of electricity even has an effect on the extraction of minerals (e.g., calcite); despite the use of fertilizers for soy production, more minerals are consumed during the life cycle of algae production. This circumstance provides a first indication of the difference in resource consumption when cultivating soy or algae.

In the drying step for soybean consumption, in general, fewer natural resources but a larger land occupation could be detected due to the use of wood chips for drying soybeans instead of natural gas in a drum dryer for algae. Furthermore, the algal biomass (10.2% DM

after dewatering) requires more drying than the harvested soybeans (82% DM), to obtain a final dry weight of approximately 89%, which facilitates the storage and oil extraction and prevents bacteria and moulds from growing and enzymes from inducing product-deteriorating chemical reactions (Ratti, 2001).

The total resource footprint of the oil and meal production step (crushing) for the soybeans and algae is very similar, especially when the results are expressed per kg meal: as calculated in section 5.2.2, the exergy content of the soybean meal is higher than that of the algal meal, which means that more algal meal must be produced to preserve the same functionality.

Table 8The resource footprint of soybean production in Brazil and transportation of soybean meal to the Netherlands is compared to the
resource footprint of algal meal production in a biorefinery concept in the Netherlands. As LCIA method, the CEENE method was used
to calculate the resource footprint of this ESA study which was based on LCA methodology. The functional units produced were 1
 MJ_{ex} (algal/soy) meal and 0.51 MJ_{ex} (algal/soy) oil. The relative contributions to the total resource footprint were calculated for the
different processes and impact assessment categories.

	Abiotic	Fossil	Nuclear	Metal	Minerals	Water	Land use	Atmos-	TOTAL	Relative
MJ _{ex} /functionalities	Renewables	fuels	resources	ores				pheric		contribu-
								resources		tion (%)
LINEAR ECONOMY										
Soybean cultivation ⁽¹⁾	1.18E-02	2.69E-01	2.79E-02	7.15E-04	6.91E-04	2.57E-02	4.81E+00	0.00E+00	5.15E+00	91.47
Drying	6.97E-04	2.33E-03	9.15E-04	2.03E-05	3.92E-05	1.76E-04	1.75E-01	0.00E+00	1.80E-01	3.19
Crushing	1.45E-02	7.76E-02	1.68E-03	1.26E-05	2.23E-05	1.28E-03	3.52E-03	0.00E+00	9.86E-02	1.75
Export to the Netherlands	2.96E-03	1.74E-01	1.04E-02	1.46E-04	7.32E-04	4.65E-03	9.29E-03	0.00E+00	2.02E-01	3.58
TOTAL	2.99E-02	5.23E-01	4.08E-02	8.94E-04	1.48E-03	3.18E-02	5.00E+00	0.00E+00	5.63E+00	
Relative contribution (%)	0.53	9.29	0.73	0.02	0.03	0.56	88.85	0.00		
BIOREFINERY										
Algae cultivation ⁽²⁾	2.04E+01	4.68E+02	5.10E+01	2.20E-01	1.53E-01	6.29E+01	1.91E+01	0.00E+00	6.22E+02	<i>99.</i> 53
Drying	7.69E-03	2.79E+00	1.39E-02	1.95E-04	2.92E-04	5.54E-03	2.54E-03	0.00E+00	2.82E+00	0.45
Crushing	2.06E-03	1.32E-01	5.07E-03	1.29E-05	2.35E-05	4.34E-03	2.10E-03	0.00E+00	1.46E-01	0.02
TOTAL	2.05E+01	4.72E+02	5.11E+01	2.20E-01	1.54E-01	6.30E+01	1.91E+01	0.00E+00	6.25E+02	
Relative contribution (%)	3.28	75.50	8.18	0.04	0.02	10.08	3.06	0.00		

⁽¹⁾ Soybean cultivation takes place in Brazil.

⁽²⁾ Algae cultivation takes place in The Netherlands. Processes included: 2nd condensing step, inoculum production, algae cultivation T1 and T2, dewatering units (coalescer and centrifuge) T1 and T2. The environmental impact of the second condensing step is included because this step is carried out entirely to support algae cultivation (allocation based on physical and chemical exergy content; 82% of the impact allocated to heat production (in the form of hot water) and 18% to the flue gases from condenser 1).

To have the soybean meal available in the Netherlands, it had to be transported over several kilometers. However, this transportation makes only a minor contribution (3.58%). When the total resource use is considered, the linear economy performs better than the biorefinery, mainly because of the high resource (energy)-demanding algal cultivation stages. The land use category contributes more to the total footprint of the linear economy with soybean production while more fossils, nuclear and abiotic resources are used within the algae production scenario (relative percentages).

The rather high differences in the resources consumed could be expected because a comparative study is conducted between a mature, large-scale technology (soybean meal/oil production) and a young, pilot-scale process chain (microalgae meal/oil production) that is still under development. As explained previously, algae have enormous potential. However, the LCA results reveal that significant improvement in terms of energy efficiency must be realized to become competitive with commonly used protein-rich feed ingredients (soybean meal).

A sensitivity analysis

The electricity production mix of the Netherlands is mainly based on fossil fuels, but there is the potential to use more renewable sources of energy, such as onshore or offshore wind energy. In this sensitivity test, the effect of changing the source of electricity production for the algal biorefinery is researched: 'electricity, at wind power plant (RER)' from the database ecoinvent version 2.2 is used. In addition, the focus here is to lower the energy consumption of mixing and blowing the flue gases into the ponds. Tests are currently being performed to modify the operating hours of the existing devices (e.g., no air sparging at night), but this change should not affect the biomass yield. For the sensitivity test, 12 hours per day of blowing and 18 hours per day of mixing is assumed. Eventually, more energy-efficient blowers can be suggested. The BOSA ventilators (capacity of 2.7 kW) could be replaced by fancom blowers (capacity of 286 W), which have a sufficient maximum flow capacity of 5240 m³ h⁻¹. Furthermore, the assumption that a higher areal biomass productivity of 6 g m^{-2} day⁻¹ can be reached (personal communication) and more biomass could be harvested (5% instead of 1.9%), possibly by small modifications to the coalescers, is made. According to the studies of Jorquera et al. (2010) and Lardon et al. (2009), even higher biomass productivities can be achieved in open pond systems (e.g., 11 g m⁻² day⁻¹ and 24 g m^{-2} day⁻¹, respectively). However, accounting for the climate conditions of the Netherlands, it is assumed that a maximum of approximately 20 ton DM ha⁻¹ year⁻¹ could be established. Genetic and metabolic engineering of the algal species could be one pathway to achieve such a yield (Christi et al., 2007). Another possibility is to alter the culture conditions, as increasing the average temperature of both the outdoor and indoor algae pond has a positive effect on the algal growth (Aleya et al., 2011; Chalifour and Juneau, 2011). However, when more hot flue gases (C-source) and hot water is pumped to the ponds, more energy use, evaporation losses and pH fluctuations will occur (which can hamper algal growth). Therefore, more experiments/trial-and-error studies are necessary to achieve optimum biomass yield at a minimal environmental impact. Detailed information about the sensitivity of the final resource footprint results with respect to each parameter can be found in Table A.9 of Appendix A. In this section, the results of a modified sensitivity test of a hypothetical algal cultivation scenario are discussed, i.e., the sensitivity of the LCA results toward changing each proposed parameter is examined. This analysis shows the potential of microalgae cultivation in a biorefinery for animal feed applications in the near future.

As shown in Figure 13, these changes lead to a considerable reduction in the resource consumption: the base case (as explained in section 5.2.1) consumes 625 MJ_{ex} to produce 1 $MJ_{ex meal}$ and 0.51 $MJ_{ex oil}$, while the sensitivity test reveals the possibility of achieving a resource footprint that is 20 times less (29.67 MJ_{ex} (1 $MJ_{ex meal}$ and 0.51 $MJ_{ex oil}$)⁻¹). When compared with soybean meal and oil production, it appears that the total footprint is still a factor of six too high to be competitive from an environmental sustainability point of view. However, when abiotic renewable sources are considered to be inexhaustible, it changes the footprint of both systems: 5.60 MJ_{ex} and 5.61 MJ_{ex} for soybean and algae production, respectively. Even some fresh water requirements could be reduced when the centrate is recycled back (perhaps after filtration to remove the possible bacteria) to the ponds. At that point, the algal meal could be produced with a similar or even lower (non-renewable) resource footprint profile compared with soybean meal (section 5.3.2).



Figure 13 Comparison of resource footprint between base case and sensitivity test scenario of algal meal production in a biorefinery concept in the Netherlands using the CEENE method for 1 MJ_{ex} algal meal and 0.51 MJ_{ex} oil.

5.4 Conclusions

An environmental sustainability assessment is performed on a linear economy and a biorefinery, both producing a basket of functionalities: 1 MJ_{ex} (algal/soy) meal, 0.51 MJ_{ex} (algal/soy) oil, 986.62 MJ_{ex} electricity, 139.15 MJ_{ex} heat and 1015.76 MJ_{ex} digestate. As can be concluded from the EA that is performed on the integrated algal biorefinery, the drying had the lowest exergetic efficiency (44%). Optimizing the harvesting and dewatering methods (four centrifuges instead of two coalescers and two centrifuges) appeared to be beneficial in terms of the biomass production, but this advantage disappeared due to additional energy consumption.

The ESA (LCA) study revealed that to produce the same functionalities, it is better to feed the livestock in the Netherlands with protein-rich soybean meal instead of algal meal from a resource point of view because algae cultivation is, for now, much more energy intensive. Especially the electricity use of blowing flue gases into the ponds has a major contribution to the total footprint, and more efficient equipment must be used.

PART III

Towards a better accounting for the environmental impact of using surface area in LCA

Chapter 6

Accounting for the occupation of the marine environment as a natural resource in life cycle assessment⁴

 $^{\rm 4}$ Redrafted from

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Taelman, S.E., Champenois, J., Edwards, M. D., De Meester, S., Dewulf, J. (2015b) Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe with a focus on quantifying sea surface occupation. Algal Research 11, 173–183.

6.1 Introduction

6.1.1 Human demand for land resources

Through human history, it has become increasingly evident that the welfare of a population depends on the availability of land and its resources. An estimation of the United States Census Bureau (USCB) revealed a global world population of 7.122 billion people in 2013 which is expected to rise, leading to an increase in consumption of resources (Livi-Bacci, 2012). When the demand for resources is outspacing the capacity of the biosphere (biocapacity), it will strongly affects the Earth's ecosystems. The biocapacity indicates the potential of land and sea areas to serve particular uses and represents the ability of the biosphere to meet the human demand for resources and waste disposal (Ewing et al., 2010b). These areas support significant photosynthetic activity and production of biomass. Less productive areas such as deserts, glaciers and the open oceans are not included. To calculate the biocapacity available per capita, the total biologically productive area (land and sea) on earth was expressed in global hectares divided by the amount of people living on the planet (Kitzes et al., 2007). Today, the available biocapacity per capita is about 1.78 global ha person⁻¹ (world average). In contrast, the global ecological footprint, which is the human demand expressed in land area, corresponds to 2.7 global ha person⁻¹. This results in a 50% overshoot (ecological deficit) (Ewing et al., 2010a).

6.1.2 Land occupation as a sustainability issue

Obviously, it is not sustainable to put such a pressure on our planet because the ability of future generations to meet their own needs is diminished (United Nations (UN), 1987). Therefore, the occupation and exploitation of productive areas is of great concern. When dealing with the sustainability of particular products that require land area during their life cycle, the most scientifically sound methodology is LCA (Koellner and Scholz, 2007).

Over the last years, it has been found important to assess the issue of land occupation and its use. For example, many renewable products (e.g., biofuels) seem beneficial for the environment in comparison with the non-renewable alternatives, except for areal land use (Delucchi, 2010). Therefore, the assessment of terrestrial land use (or land occupation) has gained attention in LCA. The use of productive land can lead to several impacts, e.g., loss of soil quality, loss of biodiversity and resource depletion (Finnveden et al., 2009). Nevertheless, only a few life cycle impact assessment (LCIA) methods, enclosing midpoint (problem-oriented approach such as climate change) and/or endpoint (damage orientedapproach such as human heath) category indicators, are available to quantify the effect of using terrestrial land resources on the environment. For example, the Soil Organic Carbon (SOC) indicator of Milà I Canals et al. (2007a) can be used to model loss of soil quality. Indicators developed to assess the impact on biodiversity are, amongst others, the Potentially Disappeared Fraction (PDF) of species from the RECIPE method (Goedkoop et al., 2009) and the Ecoindicator 99 method (Goedkoop and Spriensma, 2001). For the resource depletion aspect of land use, the CEENE method can be used as it assesses natural resource loss (Dewulf et al., 2007a; Alvarenga et al., 2013). The latter provides spatially differentiated characterization factors, which can be used to assess the impact of using land resources in different countries. From a life cycle environmental point of view, the exploitation of the productive land areas can be accounted for through the amount of biomass harvested (e.g., Cumulative Energy Demand (CED), Hischier et al., 2009 and Cumulative Exergy Demand (CExD), Boesch et al., 2007) or alternatively by assessing the area and time required to deliver a certain amount of resources, in order to avoid double counting (Dewulf et al., 2007a).

6.1.3 Accounting for marine resources in LCA

Although many LCIA methods today include the impact of terrestrial land occupation on ecosystems, none of them considers the occupation of the much larger ocean surface area (Langlois et al., 2011). Nevertheless, it is important to quantify the environmental impact of this occupation because ever more products are delivered by marine operations, such as wild and farmed fish, seaweed, wind energy and minerals from the seabed (Allard, 2009). Most of these marine operations use relatively young technology. Therefore, the use of marine resources (area and biomass) should be accounted for in LCA studies through the development of LCIA methods that can handle marine areal occupation and resource extraction.

Marine environment

In order to develop the LCIA methods, it seems necessary to understand the complexity of the marine environment, which has an average salinity of about 35g kg⁻¹ sea water (Reddy, 2001). Three vertical zones dependent upon the availability of light are usually identified: (1) the (eu)photic zone, which is the water column down to approximately 200 m depth, receives sufficient light for photosynthesis and contains nearly all primary production, (2) the aphotic below the photic zone, reaching down to a depth of about 2000 m, where light is sufficient for vision but not for photosynthesis, (3) the abyssal zone below 2000 m depth, where complete darkness takes place from a biological point. There are also several ecological regions such as the littoral zone, the benthic zone and the pelagic zone. The littoral zone is close to the shore; extends from the high tide mark to the edge of the continental shelf (coastal regions). The benthic zone contains all life in or at the seafloor, from the shore to the deep ocean and the pelagic zone is typically separated into 4 horizontal layers in the open ocean: epipelagic, mesopelagic, bathypelagic, abyssopelagic (Jain and Sharma, 2004) (Figure 14). Of these zones, the coastal one along the continental shelves is best known and most exploited commercially. It is highly biologically productive and close to the continent, allowing different activities to take place (e.g., aquaculture, tourism, fishing, oil and gas industry).



Figure 14 Zonation of the marine environment. The three large ecological regions are the littoral (organisms living near the shore), benthic (organisms living at near the sea floor) and pelagic zone (organisms living in the open ocean). The latter has 4 large subdivisions: epipelagic, mesopelagic, bathypelagic, abyssopelagic. According to the light availability, three vertical zones can be detected: photic (0-200m), aphotic (200-2000m) and abyssal (2000m-sea floor).

LCIA method to account for marine resources

To account for natural resources when occupying marine area by different oceanic activities, one extra resource category 'marine resources' was added to the original resource-based CEENE method. Concerning land area occupation, the approach of Dewulf et al. (2007a) was based on the total exergy flow from irradiance in Western European countries, of which a maximum of only 2% can be metabolized by the vegetation. The approach avoids double counting by considering only the solar exergy use of land occupation without the solar exergy embodied in the biomass produced on that land. Alvarenga et al. (2013) provided an improved approach to the CEENE method, based on a distinction between natural and human-made terrestrial land systems. In natural systems, the extraction of land resources (e.g., wood) was accounted for through their exergy content because 1) the biomass can be maintained with no or negligible human intervention prior to extraction and 2) the land surface itself was mostly just temporary occupied by mankind, i.e. the land itself is not considered occupied. Therefore, land resources from such systems can be accounted through the exergy content of the extracted biomass. On the other hand, human-made systems make use of non-productive land (e.g., infrastructure) or productive land (e.g., intensive agriculture). The output of these land uses is human-made and is not deprived from a natural system but from the technosphere. Thus, only accounting for land occupation by humans seems appropriate. However, instead of using available photosynthetic solar exergy as a proxy for areal occupation, the net primary production (NPP) of the potential natural vegetation is used because this better represents the exergy that is deprived from the natural environment. Furthermore, a site-specific approach was developed taking into consideration the spatial differentiation of land resources.

The occupation of terrestrial land is already well known and the environmental impacts can be assessed on a life cycle basis, which until now is not the case for marine areal occupation. In this study, the objective has been to create a LCIA method for marine resources (areal occupation and biomass) by using a framework similar to that proposed by Alvarenga et al. (2013). For natural marine systems, the exergy content of most of the extracted resources was quantified. For human-made systems both the exergy based spatial and temporal characterization factors for marine realms, provinces and ecoregions were calculated based on the potential net primary production (NPP) available in the photic zone. The approach used here was included in the CEENE method and applied to a detailed case study about seaweed production in NWE. This extended version of the CEENE method (i.e., with marine resources), makes it the first LCIA method able to measure the environmental resource footprint of marine areal occupation.

6.2 Materials and methods

6.2.1 Framework

The framework developed by Alvarenga et al. (2013) was applied to marine systems (Figure 15). When assessing resources extracted from natural marine systems in LCA, CFs have to be calculated. These were used in LCIA methods to quantitatively calculate the environmental impact from emissions and resources (elementary flows). Knowledge of these factors makes it possible to convert inventories to the common unit of the category indicator (ISO, 2006b). In this study, elementary flows for natural resources derived from the marine environment and the associated CFs expressed in terms of exergy have been proposed.

Processes such as fisheries can be considered as extracting resources (in this case fish) from a *natural* environment (the ocean) because no or negligible human intervention is needed to produce the amount of fish that is already present. Resources extracted from natural systems were accounted through their exergy content; for instance, the exergy of several marine seaweeds and animal species were calculated, resulting in an average CF for both organisms (see sections 6.2.2 and 6.3.1).

Marine processes such as seaweed cultivation, construction of artificial islands, oil platforms and building of windmill parks in coastal regions are *human-made*. In these cases, the infrastructure occupies and/or hinders sunlight to reach part of the water body, which lowers the NPP locally. The final products of human-made systems (electricity, seaweed, oil, etc.) cannot be accounted as natural marine resources. Instead, these processes lead to

the occupation of marine area, which is the resource actually deprived from nature. To express this in terms of exergy, the NPP of the potential natural vegetation has to be calculated because it represents the potential NPP that an area would produce under current climate without human interference (as dealt with in section 6.2.3). Global oceanic NPP was calculated on the basis of several equations which has been discussed further in section 6.2.3. Thus, potential NPP maps were produced on a yearly and quarterly basis (in mg carbon m⁻² day⁻¹). To be able to quantify how much exergy is taken from marine systems while occupying marine areas, a biomass-to-exergy conversion factor (in MJ_{ex} kg⁻¹ carbon) was calculated (section 6.3.2). This factor, in turn, was multiplied with the value of each raster point of the original NPP map. As a result, the CFs for occupying marine surface area in human-made systems could be calculated according to the exergy-based potential NPP map (MJ_{ex} m⁻² yr⁻¹).

However, an important point regarding marine area occupation has to be made. When occupying the marine surface (e.g., platforms or artificial islands), the underlying water body receives no (or a neglectable amount of) sunlight (due to scattering), which avoids (lowers) NPP. Because of this, marine surface area occupation is assumed to fully occupy the (eu)photic zone, i.e. the occupation of marine surface fully blocks sunlight penetration in the waterbody which automatically avoids natural NPP production.

However, when sea surface occupation by human-made systems does not occupy this photic zone completely, i.e. allowing sunlight to partly penetrate the water body (e.g., seaweed farming), natural NPP production is not fully deprived and an *occupation factor* α is introduced. This factor represents the NPP avoided due to the infrastructure used: (1) it occupies part of the (eu)photic zone below the surface, (2) it changes the growth environment of the phytoplankton by altering sunlight penetration (creating shadow), nutrient availability and/or optimal temperature ranges. In these situations, the α factor will be greater than 0 and less than 1, which is dealt with in the case study.



Figure 15 Schematic representation: how to account for the environmental impact of occupying human-made marine systems (left) and natural marine systems (right). The shown photic zone is the upper ocean layer where photosynthesis and thus net primary production (NPP) occurs. Left: the characterization factor (CF) is determined by the occupation of marine surface area through infrastructure, e.g. platforms, based on the potential NPP expressed in MJ_{ex} m⁻² yr⁻¹. Right: the characterization factor is determined by the exergy content of the extracted biomass resources, e.g. fish.

6.2.2 Assessment of natural marine systems

For natural marine systems where no human intervention takes place, the CF was defined as the specific chemical exergy value of the extracted resources (e.g., seaweed). Many methods are available to calculate the exergy content, such as the group contribution method, the β -low heating value method and the macronutrient method (Alvarenga et al., 2013). The first method can be used when information about the chemical compounds and their relative percentages is available (Shieh and Fan, 1982). The second method is an option given that the atomic ratios between carbon, oxygen, nitrogen and hydrogen are known, next to the lower heating value of the resource. When the composition of carbohydrates, proteins, lipids, ash and water is identified, then the last method can be used (De Vries, 1999). In this study, the composition of several aquatic plants (32 seaweed types) and animal species (fish, mollusks, crustaceans) were examined and their exergy content calculated using the macronutrient method.

The chemical composition (weight percentages) of the top 10 most captured fish species in terms of lipids, proteins, moisture and ash (amounts of carbohydrates were negligible) was given in several scientific papers (Bittar et al., 2012; Rehbein and Oeglenschläger, 2009; Sidwell et al., 1974). After rescaling, an average composition for every species could be calculated. The exergy content of the different fatty acids and amino acids in these fish was calculated using the group contribution method (FAO, 1980; Gruger, 1967; Özden and Erkan, 2011; Wilson, 2002). Then, the average weight percentages fatty and amino acids per lipid or protein were calculated between different fish species. This made it possible to calculate the average exergy value per kg protein and kg lipid. The exergy content of water was given in Table 1 in the SI of Szargut et al. (1988), which is also used to calculate the exergy value of ash (Supporting Information Alvarenga et al., 2013; assumed composition can be found in Brehmer, 2008a). In marine areas, mainly marine fish species are caught (83%) but also a lot of mollusks (8%) and crustaceans (7%), as shown in Figure 16.

Data about the chemical composition of several crustaceans and mollusks were gathered from the study of Pisal et al. (2007) and Sidwell et al. (1974). In addition, the chemical composition (weight percentages proteins, sugars, fibers, lipids and ash) of 32 seaweed species was obtained (Ahmad et al., 2012; Hélène Marfaing, personal communication). An average moisture content of 85% was assumed to calculate the CF for fresh plant material (Ahmad et al., 2012).



Figure 16 Capture production in marine fishing areas over the world in 2010, relative percentages (FAO, 2010)

6.2.3 Assessment of human-made marine systems

In human-made marine systems (e.g., seaweed production area or aquaculture), the CF was determined by the occupation of sea/ocean area and not by the produced and extracted resources, which follows the framework of Alvarenga et al. (2013). The potential NPP was used as a basis to assess the environmental impact of occupying marine systems. By using several equations and data sources (Appendix B, Figure B.1), global NPP maps were produced on a yearly and quarterly basis, expressed in mg carbon m⁻² day⁻¹. To know how much exergy was deprived from marine systems while occupying these areas, a biomass-to-exergy conversion factor was calculated, based on the biochemical data of Hedges et al. (2002) for marine phytoplankton. Samples were collected at different marine locations and the weight percentages of carbon, hydrogen, oxygen and nitrogen were calculated. In this study, the β -low heating value method was used to calculate the exergy content of the plankton in every sample.

Apart from the temporal differentiation, also a spatial differentiation was made because NPP seems to be high, especially in the coastal regions. The reason can be found in the upwelling of water from the deep ocean. Furthermore, the input of nutrient from land or rivers contributes to enhanced NPP in these areas (Robinson and Brink, 2005). To calculate site dependent CFs, the Marine Ecoregions of the World (MEOW) system was used (Spalding et al., 2007) where 12 realms, 62 provinces and 232 ecoregions have been defined

with emphasis on coastal and shelf waters. Figure 17 displays the localization of the 12 different coastal realms and for each realm, a CF was calculated. To provide an indication about the CF for open oceans (coastal regions excluded), a 13th realm was added.



Figure 17 World map with indication of 12 coastal realms (Spalding et al. 2007) and 1 open ocean realm used to calculate the site-specific characterization factors for marine area occupation.

Net primary production (NPP) of the marine environment

Understanding of the marine environment is required in order to quantify NPP. Actively photosynthesizing microscopic plants, also referred to as microalgae, can be found in the upper sunlit layer of all oceans. This phytoplankton biomass, together with seaweed and other marine plants, forms the basis of the aquatic food web because they are photo-autotrophic: they use solar energy to convert dissolved inorganic carbon dioxide or bicarbonate ions into organic carbon which can be accessible to diverse heterotrophs (from bacteria and protists to animals). Phytoplankton are, thus, a key player in the ocean productivity cycle. A part of the produced organic carbon is respired by autotrophs for their maintenance. The other share leads to biomass growth and refers to 'net primary production' (NPP), which represents the basic biomass resource of the marine ecosystem and its food web. Therefore, NPP is the natural resource which by definition is deprived. For more information on NPP and the food web cycle, see Appendix B, Figure B.1.

NPP can be limited by two key factors: nutrient and light availability. According to the Redfield ratio C:N:P of 106:16:1, rather uniform nitrogen and phosphorus requirements for phytoplankton biomass production are distinguished (Redfield, 1958). Also, minimum amounts of iron and silicon are essential to prevent a reduction of NPP. In the deep ocean layers there are sufficient amounts of these nutrients available, but for photosynthetic organisms such as phytoplankton it is also important to have access to sufficient light. The upper zone of the marine system in which enough light can penetrate to allow photosynthesis is called the euphotic zone. This layer may range from about 10 to 200 meters depth (euphotic depth), simplified to a boundary of 1% of surface irradiance. Because of the absorption of light, the ocean's upper layer is usually warmer than the underlying deep ocean; a thermocline exists. This yields a nutrient-limited warm upper layer and a light-limited cold deeper layer. Wind or other energy sources are required to drive mixing across the thermocline, reintroducing dissolved nutrients to the euphotic zone. As a result, the highest NPP region is found where there is large nutrient supply from below and adequate light for photosynthesis (Sigman and Hain, 2012).

NPP calculations and data inventarisation

The (Eppley-) VGPM model

Datasets about global NPP in marine systems are available from the ocean productivity website of the Oregon State University. The standard Vertically Generalized Productivity Model (VGPM) was used to estimate the ocean's primary productivity, displayed as world maps (Behrenfeld and Falkowski, 1997; Robinson, 2010). This model makes use of ocean biological and physical data (chlorophyll concentration, photosynthetic active radiation, sea surface temperature) derived from satellites such as SeaWiFS and aqua MODIS. For these variables, data of the year 2012 was downloaded and used in this study. In the VGPM, the optimal productivity rate (Pb_{opt}) is the maximum observed photosynthetic rate within the water column (mg C fixed mg chlorophyll⁻¹ h⁻¹) and is modeled as a polynomial function of sea surface temperature (SST). However, in this function the photoacclimation process found in all oceanic plants was not taken into account. This process explains the fact that plants grown at low light conditions generate more pigments to capture the available light than plants grown under strong light. Consequently, the photosynthetic efficiency per pigment under high light conditions (which is correlated to high SST) is

highest, which results in an exponential curve. Therefore an Eppley-VGPM model was developed based on an exponential Pb_{opt} function which includes photoacclimation (Eppley, 1972). This curve was normalized by Antoine and Morel 1996.

In the Eppley-VGPM model, the NPP (mg C m⁻² day⁻¹) is calculated according to equation 13: NPP = [chlor.] \cdot Pb_{opt} \cdot daylength \cdot f(par) \cdot z_eu (13)

Here, [chlor.] is the phytoplankton based chlorophyll a concentration (mg chlor. m⁻³), which is derived from the aqua MODIS satellite, Pb_{opt} is the maximum carbon fixation rate within a given water column (mg C mg chlorophyll⁻¹ h⁻¹) (Eppley, 1972), the daylength is the number of hours of day light spatially differentiated (hours day⁻¹), f(par) is the photosynthetic active radiation (PAR) dependent function (/) (Behrenfeld and Falkowski 1997), z_eu represents the euphotic depth equation based on surface chlorophyll concentrations (m) (Morel and Berthon, 1989).

Enhancement of the Eppley-VGPM model

In this study, we have tried to further develop this version of the model by using the same overall NPP calculation (equation 1) as used in the Eppley-VGPM model but data for calculating Pb_{opt}, daylength and z_eu were obtained from more recent studies. The Pb_{opt} function was enhanced by improving the SST function and by adding a nutrient limitation term. This approach is based on the Monod growth rate equation (h⁻¹) which can be adapted for algal growth: $\eta = \eta_{max} \cdot [S/(K_s+S)]$ where η_{max} is the maximum algal growth rate (h^{-1}), S is the concentration of the limiting nutrient (mg dm⁻³) and K_s the half saturation coefficient (mg dm⁻³) (Goldman and Carpenter 1974). For the calculations of η_{max} , the highorder polynomial Pb_{opt} proposed by Behrenfeld and Falkowski (1997) was used because this equation gives a better relationship between actual measurements and modelling, compared to the maximum algal growth rate equation proposed by Eppley (1972). In marine systems, nitrogen (especially nitrate) is used by phytoplankton and is the main limiting nutrient in marine systems (FAO, 1987; Tyrell, 1999). In this study, the Monod equation was applied to the nitrate concentration in marine surfaces (downloaded from the World Ocean Atlas 2009 website, NOAA). The half saturation constant (K_s) of 0.5 µmol m^{-3} for phytoplankton growth versus NO_3^{-} concentration, was applied (Tyrell, 1999).

For the euphotic zone depth calculations, the 4th order polynomial of Morel and Maritorena (2001) was used for computational convenience; it is based on the results of a previous study of Morel and Benthon (1989). This is a log-log equation where z_eu is calculated based on the total pigment content within the euphotic layer (C_{tot}). This pigment content is calculated according to following equation: C_{tot} =40.6 · [chlor.]^{0.460}(Morel and Berthon, 1989).

The yearly average global daylength was fixed at 12 hours per day for every latitude. When considering seasonal variation, daylength is no longer a fixed average. In this study, the hours of sunlight available per day at any location was calculated because no useful global datasets or maps seemed to be available on a quarterly base. The CBM model described of Forsythe et al. 1995 was used to calculate daylength which is determined by the period between sunset and sunrise, latitude/longitude coordinates and the day of the year compared to the astronomical almanac. The mathematical software MATLAB version 8.1 was used to process the huge amount of 2,332,800 data points. First the daylength for every month was calculated followed by datasets per quarter, both spatially differentiated (grid size of 0.72m times 0.72m).

To make a temporal differentiation, the global annual and quarter based NPP was calculated. Datasets of the variables such as PAR, chlorophyll concentration and SST, provided by the Ocean Color website (NASA), were available per year, month or quarter. We have defined and standardized the 4 quarters among regions and variables: 1st quarter (combined data for the months March, April and May), 2nd quarter (June, July and August), 3rd quarter (September, October and November) and 4th quarter (December, January and February). To calculate the global NPP per quarter, the same equations as described previously were used but now based on datasets for each quarter. The ArcMap 10 software was used to create the different rasters and shapefiles, and tools such as raster calculator, zonal statistics and marine geospatial ecology tools were used. A schematic overview of the equations and data sources used is shown in Appendix B, Figure B.2. For this framework one should multiply this NPP amount with the occupation factor (see section 6.2.1). This occupation factor represents the part of the euphotic zone that is occupied. In this study it is considered to be equal to 1.

6.3 Results and Discussion

6.3.1 Characterization factors for resource extraction from natural marine systems

To account for the resources extracted from the natural marine system, the exergy content of the resource in question has to be calculated. According to the FAO yearbook of 2010, the top 10 fish species most captured in natural marine systems are Peruvian anchovy (Engraulis ringens), Alaska Pollock (Theragra chalcogramma), Skipjack tuna (Katsuwonus pelamis), Atlantic herring (Clupea harengus), Chub mackerel (Scomber japonicus), Largehead hairtail (Trichiurus lepturus), European pilchard (Sardina pilchardus), Japanese anchovy (Engraulis japonicus), Yellowfin tuna (Thunnus albacares) and Atlantic cod (Gadus morhua). As a result, the exergy content (CF) of the 10 most commercial species (weighted average of catch) was calculated per kg wet and dry weight. The second and third most important marine species (Figure 16) in terms of catch are crustaceans and mollusks. The CF for these species was calculated in terms of fresh and dry weight (Table 9). To represent marine seaweeds, data for the chemical composition of 32 seaweed types was gathered and the average CF obtained (Table 9). More information about the detailed calculations can be found in Appendix B, Tables B.1-B.9. Among the marine organisms in Table 9, seaweed exhibit the lowest average exergy content because, on average, only 1.15% of their dry weight consists out of lipids and as can be seen from Table B.5 of Appendix B, lipids have the highest exergy content. For fish, mollusks and crustaceans, only a neglectable amount of carbohydrates could be detected, implying there is predominance of lipids and proteins (with higher exergy contents).

Table 9	Average characterization factors (expressed in MJ exergy per kg fresh or dry
	weight) and standard deviation (Std) for fish, mollusks, crustaceans and seaweed
	products in natural marine systems.

MJ _{ex} kg⁻¹ FW*	Std (-)	MJ _{ex} kg⁻¹ DW*	Std (-)
6.6	1.65	25.6	2.10
3.9	0.53	23.4	0.61
5.0	0.38	24.0	0.69
2.4	0.23	15.3	1.53
	MJ _{ex} Kg ⁻ F W 6.6 3.9 5.0 2.4	MJ _{ex} kg · F W Std (-) 6.6 1.65 3.9 0.53 5.0 0.38 2.4 0.23	MJ_{ex} kg $^{-}$ F W $^{+}$ Std (-) MJ_{ex} kg $^{-}$ DW $^{+}$ 6.6 1.65 25.6 3.9 0.53 23.4 5.0 0.38 24.0 2.4 0.23 15.3

* FW= fresh weight, DW= dry weight

The calculated CFs make it theoretically possible to assess the extraction of these natural resources from an environmental point of view. In practice, LCA databases such as ecoinvent should provide reference flows related to these natural resources (e.g., fish, seaweed, etc.) from the marine environment. These reference flows can then be used as proxies for some marine processes, for which we cannot calculate the resource footprint yet. For example, the ecoinvent (v2.2) database contains only a reference flow for fish (*fish, unspecified, in sea*') which could be a natural resource input for fisheries (process not yet available in ecoinvent) (Weidema and Hischier, 2010). Therefore, the authors of this manuscript advise to (1) collect more information about marine processes and (2) change the reference flow to '*fish, unspecified, in natural marine systems*'. In addition, new reference flows for mollusks, crustaceans and marine aquatic plants are proposed: '*crustaceans, unspecified, in natural marine systems*', 'mollusks, unspecified, in natural marine systems' and 'seaweeds, unspecified, in natural marine systems', respectively. This is recommended as it is the only way to apply the marine resource-based LCIA method proposed in this study to the ecoinvent database.

6.3.2 Characterization factors for occupying human-made marine systems

CFs for marine occupation by human-made systems were obtained on the basis of the potential NPP available in marine systems. A potential NPP map (mg C m⁻² yr⁻¹) was produced and a biomass-to-exergy conversion factor calculated. A range of 41.49 – 41.62 MJ_{ex} per kg carbon fixed could be indicated with an average of 41.55 MJ_{ex} kg C⁻¹ and a coefficient of variation of 0.001. Detailed information on this calculation is given in Appendix B, Table B.10. The conversion factor was used to multiply each value of the potential NPP map, now expressed in terms of exergy (Figure 18).



Figure 18 World map of characterization factors of marine area occupation in human-made systems, based on the potential net primary production available ($MJ_{ex} m^{-2} yr^{-1}$). The continents are presented in black.

Spatial distribution

Site-dependent CFs for human-made systems were calculated for the 12 coastal and 1 open ocean realm (Table 10). The Southern ocean near Antarctica yielded the smallest average CF, in contrast to the CF of the temperate northern Pacific (factor 10), located at the east coast of Asia, due to the high sea surface temperature differences. A total area of 359,436,143 km² of marine systems was covered, representing 1.65·10¹⁵ MJ exergy NPP, of which 51% is allocated to the open oceans. Other big contributors are the temperate northern Pacific and Atlantic. Unlike these last two, which exhibit the highest mean potential NPP, the open ocean is strongly contributing to the total potential NPP available in marine systems by its large surface area.

For the moment, mostly coastal areas are exploited and occupied. As can be seen from Table 10, most of these areas (Artic, temperate Australasia, temperate northern Atlantic and Pacific and temperate southern America and Africa and the tropical eastern Pacific) have a much higher mean potential NPP than open oceans and occupying these regions can therefore lead to high environmental impacts. Though, when comparing the average potential NPP for marine systems (4.59 MJ_{ex} m⁻² yr⁻¹) with that of terrestrial systems (21.5 MJ_{ex} m⁻² yr⁻¹, Alvarenga et al., 2013), it seems more environmental damaging to occupy terrestrial land instead of marine surface as biology is concerned. The Appendix B provides

more details about the differences between the northern and southern hemisphere (Tables B.17 and B.18). Site-dependent CFs for marine areal occupation per province or ecoregion as defined in Spalding et al. 2007 can be found in Appendix B, Tables B.11 and B.12.

Table 10Site-dependent characterization factors for marine area occupation in human-made systems. For 13 realms, the area
(km²), mean potential NPP (MJ_{ex} m⁻² yr⁻¹), minimum (min) and maximum (max) NPP (MJ_{ex} m⁻² yr⁻¹) and standard deviation
(std) was calculated. The total NPP per realm and for the entire marine system is shown in column 8 and the relative
percentages of each realm in column 9.

	Zonal	Area	Mean potential	Min	Max	Std	NPP per realm	Relative
Realm	code		NPP					percentage
	(-)	(km²)	(MJ _{ex} n	n ⁻² yr ⁻¹)		(-)	(MJ _{ex} realm ⁻¹ yr ⁻¹)	(%)
Arctic	9	12,009,906	10.12	0.00	141.64	7.89	1.21 x 10 ¹⁴	7.37
Central Indo-Pacific	7	27,827,840	1.92	0.00	73.12	2.24	5.35 x 10 ¹³	3.25
Eastern Indo-Pacific	12	18,974,518	1.67	0.03	23.75	1.71	3.17 x 10 ¹³	1.93
Open ocean	13	223,684,180	3.72	0.00	71.21	3.83	8.33 x 10 ¹⁴	50.53
Southern Ocean	4	10,322,300	1.51	0.00	19.96	1.74	1.56 x 10 ¹³	0.95
Temperate Australasia	10	5,654,772	5.48	0.81	135.71	2.47	3.10 x 10 ¹³	1.88
Temperate Northern Atlantic	8	9,854,227	15.96	0.54	192.74	13.06	1.57 x 10 ¹³	9.55
Temperate Northern Pacific	2	9,653,781	16.18	0.88	262.02	9 . 85	1.56 x 10 ¹³	9.48
Temperate South America	6	6,036,587	10.60	0.45	257.38	9.71	6.40 x 10 ¹³	3.88
Temperate Southern Africa	1	1,986,349	14.48	2.18	136.18	14.44	2.88 x 10 ¹³	1.75
Tropical Atlantic	3	13,933,694	4.87	0.17	253.35	9.06	6.79 x 10 ¹³	4.12
Tropical Eastern Pacific	11	4,185,132	7.04	0.63	120.33	5.78	2.95 x 10 ¹³	1.79
Western Indo-Pacific	5	15,312,855	3.81	0.00	82.96	5.79	5.83 x 10 ¹³	3.54
Marine system		359,436,143	4.59				1.65 x 10 ¹⁵	

Temporal distribution

The resource footprint of marine areal occupation may differ in time. This is relevant for processes related to short period occupations, e.g., aquaculture trials. Because NPP has a doubling time of a few hours or days, depending on the environmental conditions and type of phytoplankton, it is unlikely that the occupation of marine surface area in a certain quarter would affect the NPP production in other quarters (Malcata, 2011). Therefore, a temporal distribution of the CFs was made (Table 11). Potential NPP world maps per quarter ($MJ_{ex} m^{-2} yr^{-1}$) and local CFs per quarter (based on realms) can be found in Appendix B, Figures B.3-B.6 and Tables B.13-B.16, respectively. Because the global CFs are world averages, it might be interesting to know the difference in marine NPP for the northern and southern hemisphere (Table 11). For the 1st and 2nd quarter, more NPP can be expected in the northern hemisphere, whereas NPP is highest in the southern hemisphere for the 3rd and 4th quarter. The difference of the CFs per quarter between both hemispheres is a result of the geographical variation of the amount of daily lit hours (Appendix B, Tables B.17 and B.18).

Table 11 The quarter-dependent characterization factors for marine area occupation in human-made systems. The mean potential NPP was calculated $(MJ_{ex} m^{-2} yr^{-1})$ per quarter for each hemisphere and as a world average.

	Mean potential NPP (MJ _{ex} m ⁻² yr ⁻¹)						
	Northern Southern Global						
Quarter	Hemisphere	Hemisphere					
1 st (March-April-May)	5.84	3.92	4.81				
2 nd (June-July-August)	6.96	3.51	5.11				
3 rd (September-October-November)	4.04	4.62	4.36				
4 th (December-January-February)	3.51	5.18	4.49				

6.4 Case study

To illustrate the methodology developed in this study, a detailed case study on the environmental resource footprint of two seaweed cultivation systems in North West Europe is presented because aquacultural activities may cause shading in the photic zone which prevents NPP production by photosynthesis (Langlois et al., 2014b).
6.4.1 Background

As potential economic and ecological benefits of seaweed production become apparent, a wave of interest from government, research institutions and industry has developed over the last few years, which is translated in high aquaculture production rates of 20.8 million tonnes (wet weight) in 2012 compared to 6.4 million tonnes in 2000 (Figure 19) (FAO, 2012; Kilinç et al., 2013). The majority of seaweed (99% in 2012) is produced on a commercial scale in Asian countries, especially in People's Republic of China (54%), Indonesia (28%), the Philippines (7%) and North and South Korea (4%), where relatively low-technological business provides income, employment and foreign trade (FAO, 2012; Pickering, 2006). These countries have a long history of eating a wide variety of seaweeds including Pyropia and Porphyra spp., Laminaria spp., Saccharina spp. and Undaria pinnatifida. In total, 66% of the worldwide seaweed production is produced for the food industry. EU imports of seaweeds have traditionally been used by the pharmaceutical, cosmetic and food industry for their useful extracts (e.g., phycocolloids such as agar) or as products for agriculture (fertilizer, animal feed) and are less commonly used for direct human consumption (Ngo et al., 2011). Compared to Asia, seaweed production in Europe is still small in scale and can be found in countries such as France, Spain, Portugal, Ireland and Norway, amongst others, either as commercial or experimental setups. The main cultivated species to date are Saccharina latissima (sugar kelp) and Undaria pinnatifida (Wakame) (NETALGAE, 2012).



Figure 19 Worldwide aquaculture production of aquatic organisms and seaweed (million tonnes) between 2000 and 2012 (FAO, 2012)

Because of the high population density in Europe, there is greater competition for land arising from the growing demand for food, energy and accommodation. Therefore, seaweed cultivation in European seas could be a solution to reduce the pressure on land and its resources. As seaweed cultivation and the search for markets in Europe is still in its infancy, an estimation of the environmental sustainability may assist in their further development. The CEENE 2014 method (developed in this chapter) is used to determine the environmental resource footprint of seaweed production in European coastal areas, in particular in the Atlantic Ocean on the West coast of Ireland and the Northern coast of France (Brittany). Different seeding procedures and nearshore cultivation systems were applied (single longline system in Ireland versus raft system in France). To be able to quantify all resources used (also marine resources), the occupation factor α (as described in section 6.2.1) was calculated for both production sites.

To summarize, the objectives of this case study are fourfold: 1) Examine environmental sustainability in terms of natural resource use of seaweed production at different production regions NWE, 2) Provide a case-study in which sea surface occupation factors α are calculated (Taelman et al., 2014), 3) Compare the resource footprint of seaweed with that of microalgae and different types of terrestrial biomass (maize, potatoes and sugar beet) and 4) Analyze feasible options to improve the footprint of seaweed production in NWE.

6.4.2 Materials and methods

Process description

Within the context of the INTERREG IVB NWE EnAlgae project, *Saccharina latissima* was cultivated near the coasts of Ireland and France as the cold-water climate of European seas is particular suitable for brown algae. The seedling production in the hatcheries and the nearshore site requirements are discussed below. As seaweed production in Europe is still in its initial stage and data regarding processing of the biomass towards a final application is scarce, focus should be first on optimizing the cultivation processes.

Seaweed cultivation in Ireland

Seedling production in the hatchery

In the West of Ireland, the National University of Ireland, Galway (NUIG) operates an aquaculture research facility (The Ryan Institute Carna Research Station) in Carna, Co. Galway (Appendix C, Figure C.1). The facility is located at a local pier, and has a complex water treatment system installed (Appendix C, Figure C.2) that can supply seawater for use in large-scale experimental flow-through and recirculation systems. The facility currently operates an Atlantic cod (*Gadhus morhua*) breeding programme and a seaweed hatchery facility amongst other experimental research programmes.

Seawater is supplied by 2 fibroc centrifugal pumps of 15 kW (one operating and one standby) at a continuous mode with a speed of approximately 21 L s⁻¹. A Liquivac Priming pump is necessary to start the 2 main Fibroc pumps. Further, the water passes through a Bernoulli pneumatically controlled filter system (250 μ m) to remove suspended solids and compressed air is delivered by Atlas Copco compressors. There are 2 filters and 2 compressors available, but only one of each is on duty at the time. Most of this filtered seawater is used in the fish breeding units, only a small part (about 45 m³ year⁻¹) is pumped to the seaweed hatchery facility tanks where the water quality is further improved by 2 inline TMC cartridge filters of 10 μ m and 1 μ m mesh size, running under pressure from the incoming water, and a TMC UV sterilizer to eliminate harmful microorganisms. Seawater required for flask culture of gametophytes is sent to an Astell autoclave for complete sterilization (Appendix C, Figure C.2). Flasks are cleaned using a phosphate-free laboratory detergent (Decon 90) in advance of seawater sterilisation.

The production of seedlings starts with the collection of fertile *Saccharina latissima* in the lower intertidal and subtidal coastal zones (Appendix C, Figure C.3). In the hatchery (120 m²), the reproductive sori, which are clusters of sporangia containing many millions of zoospores, are cut out from the blade of seaweed, cleaned and air-dried (24 h) before being placed in small flasks (6 L) with autoclaved water. The numerous flagellated male and female zoospores (haploid) that are released after this process develop into male and female gametophytes (also haploid). Under laboratory conditions, the gametophytes

produce gametes and a fusion between a male and female gamete leads to a diploid zygote that develops into juvenile sporophytes, or seedlings. Light intensity and spectra is an important parameter during these reproductive phases; red/white light is used at the beginning and blue light is required for gametogenesis later on (Edwards and Watson, 2011). The sporophyte culture is sprayed on 12 collectors (each containing 50 m of polyvinyl alcohol fibers or culture string). These collectors are placed in 5 culture tanks filled with UV-sterilized seawater for at least a month. F/2 culture medium containing nitrates (NaNO₃) and phosphates (NaH₂PO₄.H₂O), vitamins (B1, B12 and H) and trace elements (e.g., FeCl₃), is used in the flasks and tanks (Harrison and Berges, 2005). The cultures are kept at 10 °C using a room air chiller, which is the optimum temperature for all life cycle stages. Agitation and aeration is provided by a blower device operational 24 hours a day (one in standby). Approximately three batches (of ± 5 weeks) per year provides 9000 m of seeded cultivation string in total.

All wastewater from the hatchery is collected and pre-filtered in a hydrotech drumfilter. A Grundfos pump is installed for backwashing. Suspended solids are removed in order to improve the efficiency of the Wedeco UV disinfection unit where bacteria and viruses are destroyed by high intensity UV (Appendix C, Figure C.2).

Grow out phase in the sea

The seaweed grow out phase is located in the South West of Ireland within Ventry Harbour, Co. Kerry and is owned and operated by the commercial seaweed farm Dingle Bay Seaweeds (Castletownbere, Co. Cork) (Appendix C, Figure C.1). In total, 18 hectares of sea site is licensed for seaweed aquaculture (Appendix C, Figure C.5). Therefore, a van is used to transport the seeded collectors between the hatchery and Ventry Harbour. Other equipment (e.g., culture rope, anchor chains, buoys) is transported from Castletownbere to Cuan Pier, Ventry Harbour by lorry and from Cuan Pier to the seaweed site by several boats. These boats were also used for maintenance and harvest of *Saccharina latissima*.

In December, the seeded seaweed collectors are wrapped around the culture rope (280 m per longline) at deployment. Figure 20 gives a schematic representation of the equipment used at sea to cultivate *Saccharina latissima*; 4 anchors and 6 large buoys are used for 3

longlines. Manual harvesting takes place in May, when the quality of the seaweed is optimal. About 25 kg seaweed (9.7% DM) per m longline can be harvested.



Figure 20 Equipment for nearshore seaweed cultivation in Ventry Harbour, Co. Kerry, Ireland. A cultivation unit contains 3 longlines, 4 anchors and anchor chains, 8 anchor ropes and buoyancy is required to maintain the longlines at 0.5-1m below the water's surface.

Seaweed cultivation in France

Seedling production in the hatchery

The seaweed hatchery of 165 m² in France is located in Pleubian, at the CEVA (Centre d'Etude et de Valorisation des Algues) facility (Appendix C, Figure C.1). Seawater is pumped (1.5 kW, 12 m³ h⁻¹) from the sea to a 20 m³ storage tank. A second pump (0.55 kW, 4 m³ h⁻¹) is used to deliver water at the hatchery. Seedling breeding starts with the collection of local fertile *Saccharina latissima* (Appendix C, Figure C.4). Sori are cut from the blades and gently brushed to remove animals and epiphytes. Autoclaved seawater is used for the preparation of fertile material (autoclave specification: SMI AVX 5091). Further, the cleaned seaweed material is stored in dark conditions at 10-15°C for one night to dehydrate the seaweed pieces. On day 2, the fertile seaweed parts are submerged in autoclaved seawater to release the spores (spore solution).

The seedlings are produced in filtered seawater (2-step filtration procedure using 10 μ m and 1 μ m HydrexTM filter cartridges). Because of membrane fouling, the cartridges are changed every year. When the seawater is poured into the tanks, sodium hypochlorite is added to sterilize and sodium thiosulfate is used to neutralize the bleach. Afterwards, the spore solution is poured into 2 cultivation tanks (2 batches per year). A blower device (operational 24 hours a day) is used to provide mixing and aeration. As nitrogen and phosphorous source in the culture medium, NH₄NO₃ and PO₄HNa₂.2H₂O is used, respectively. Compared to the seeding procedure at NUIG, there is no gametophyte development in CEVA. Direct seeding of mobile zoospores is cheaper (no maintenance of the immobile gametophyte phase), but, as a disadvantage, more fertile sporophyte is necessary because of the avoided gametophyte step. On a yearly basis, 4000 m of cultivation string is seeded, i.e. 80 collectors are prepared. The development of seedlings under controlled conditions lasts for about 5 weeks, until they reach 3-5mm in length. Each collector contains 60 m of string, which is wrapped around 50 m of culture rope. This procedure takes place in the hatchery, not at sea.

Grow out phase in the sea

Around mid-December, the nursery culture is transferred to the sea. The sea farm is located 2 km from shore and 8 km from the nearest harbour of Lézardrieux (use of boat and lorry is required) (Appendix C, Figure C.1). Because of the many surface currents at the sea site, a raft system was chosen over a longline system. The main reason behind this choice is the lower areal yield of a longline system under these conditions because a large distance between the ropes is required to avoid friction.

Figure 21 illustrates the raft system used for the grow out phase of *Saccharina latissima*. In total, about 6 hectares of sea site is licensed for CEVA to use. There are two types of raft systems (for experimental reasons), and 4 raft units available. The 1st type is 20 m x 50 m with 11 longlines (2 units) and the 2nd type is 20 m x 100 m with 11 longlines (2 units). These cultivation units occupy 6000 m² of sea surface and about 2.85 ha of sea site (taking into account anchoring and space between the units). A schematic representation of the sea farm is shown in Appendix C, Figure C.6. Each raft system contains 2 main HDPE tubes at each side, some intermediate HDPE tubes (2 tubes of 10 m in type 1, 6 tubes of 10 m in type 2), 2 anchors, 4 anchor blocks, 2 buoys of 1000 L and 2 buoys of 300 L. The longlines

are at 1.5 m depth below the sea surface. In total, 3300 m culture rope is harvested manually in May, keeping in mind that harvesting in June yields more biomass but, due to more epiphytes, food and feed applications are limited. A maximum yield of 20.3 kg fresh weight (FW) m⁻¹ could be achieved with this system but due to friction of culture ropes with buoys and tubes the yield drops to an average of 5 kg seaweed (9.7% DM) per m longline.



Figure 21 Schematic representation of a raft system (20m x 50 m, type 1) and anchoring used at the sea farm (6000 m²) near the coast of Lézardrieux, France. Only 2 culture ropes (1.5m below the sea surface) are shown.

Life Cycle Assessment (LCA)

An environmental LCA was carried out according to the ISO standards 14040 and 14044 (more information available in section 2.1).

Goal and scope and inventory analysis

Comparing the environmental sustainability in terms of natural resource use for two different seaweed production scenarios in North West Europe requires a common functional unit and system boundaries. In this study, results are expressed per MJ_{ex} *Saccharina latissima*. Data on the composition was obtained from CEVA and was assumed to be applicable for both cases (Appendix C, Table C.1). Processes related to the seaweed hatchery and grow-out phase at sea are included in the foreground system. A schematic representation of the foreground system and background system can be found in Figure 22.



Figure 22 Schematic representation of the foreground (red dotted line) and background system (green dotted line) of both seaweed production systems located in Ireland and France.

All processes related to seaweed production are taken into account; e.g., illumination of juvenile sporophytes, cooling of the seaweed hatchery rooms, fresh water use, air sparging in the cultivation tanks, transport of the ropes to the sea site, the use of boats for maintenance and harvest of the biomass and land (hatchery) and sea surface (grow-out phase) occupation. Data related to the foreground system is collected at the production sites (Appendix C, Tables C.3 and C.4). Potential emissions at the foreground system were not quantified. Data about the products and processes from the supply chain (i.e. background data) were selected from the ecoinvent version 2.2 database (Frischknecht and Rebitzer, 2005). This LCA study applied a cradle-to-gate boundary.

In the case of Ireland, the impact of using electricity and infrastructure during pre- and post-treatment of seawater is allocated to the volume of water used within the hatchery. The main part of the seawater (99%) is used for breeding fish instead of seaweed. In the hatchery, a blower provides aeration and mixing in the seaweed tanks but this device is also used for aeration in other (fish) tanks. Therefore, the electricity consumption of the blower is allocated on the basis of the volume of the seaweed tanks (which represents only 0.05% of the total volume). In France, the blower in the hatchery supplies air for microalgae (330L) and macroalgae (600L) cultivation, i.e. the same allocation method as in the case of Ireland (volume-based) was applied.

In section 6.4.3, the resource footprint of seaweed production is compared to the cultivation of marine microalgae and some terrestrial plants (sugar beet, maize, potatoes). Inventory data related to *Nannochloropsis* sp. production was available in Taelman et al. (2013). Database ecoinvent version 2.2 provided data for the terrestrial plants (Frischknecht and Rebitzer, 2005). Table C.2 of Appendix C, shows the chemical composition and exergy content of the terrestrial plants (Brehmer et al., 2008b).

Impact assessment

Resource accounting method

In this study, the environmental impact assessment was performed based on the CEENE (2014) method, which is an exergetic LCIA method that quantifies the impact on the environment through the extraction and/or consumption of several natural resources, including marine resources (as described earlier). The collection of fertile Saccharina latissima at both sites was accounted for through the exergy content of the seaweed biomass; 13.44 MJ_{ex} kg⁻¹ seaweed DW (Appendix C, Table C.1). Spatially differentiated characterization factors (CF) were used for the occupation of terrestrial land (Alvarenga et al., 2013) and marine sea surface (Taelman et al., 2014). The CFs for direct land occupation in Ireland and France are 25.7 MJ_{ex} m⁻² yr⁻¹ and 28.0 MJ_{ex} m⁻² yr⁻¹, respectively, and for direct marine sea surface occupation 22.7 MJ_{ex} m⁻² yr⁻¹, as both nearshore regions are located in the Celtic Sea. When compared to the production of microalgae and some terrestrial plants, different CF's for land occupation were used (26.9 MJ_{ex} m⁻² yr⁻¹ for Belgium and for 24.4 MJ_{ex} m⁻² yr⁻¹ for Switzerland). For the upstream processes (background system), about 95% of the occupied land has its origin in Europe and no sea surface occupation is considered. Therefore, the impact of land occupation in the background system is calculated according to the average CF of Europe (23.2 MJ_{ex} m⁻² yr⁻¹).

Marine sea surface occupation factor α

An important point regarding marine area occupation compared to land occupation is the three-dimensionality of the ocean. NPP production is possible in the upper layers of the water body, i.e. the photic zone, where sufficient sunlight can penetrate to stimulate growth. An occupation factor α was introduced to deal with the possibility of occupying only part of the photic zone. For example, nearshore seaweed farming on one hectare sea surface still allows sunlight to penetrate the waterbody due to the longline/grid structure used for cultivation. In this study, the α factor is calculated for the infrastructure used at the West coast of Ireland and the Northern coast of France. These coastal areas provide sufficient nutrients (the coast of Brittany in particular provides nutrients in excess, therefore sunlight availability may be regarded as the limiting factor for NPP production (Morand and Merceron, 2005; Cave and Henry, 2011; Connolly et al., 2009).

In Ireland and France, the cultivation systems occupy a total sea surface area of 176 400 m² and 6000 m², respectively. However, this is not necessarily a full occupation because of the open/grid structure, i.e. sunlight can still penetrate the surface between the culture ropes which allows natural NPP production. At the time of deployment (December 15th), the effective sea surface occupation (culture ropes, HDPE tubes, buoys, etc.) amounts to 1180 m² and 336 m² for Ireland and France, respectively. The culture ropes are positioned parallel to the direction of the water movement (Figure 23).



Figure 23 Modeling principle sea surface occupation related to seaweed growth on culture ropes positioned parallel to the direction of the water movement at sea.

It is assumed that the seaweed blades float under an angle of 20° (estimation based on sea site visits). The length of the blades can be considered as the hypotenuse A of a right triangle. A standard cylinder, with a culture rope as its axis, represents the seaweed biomass. The radius of the cylinder becomes larger as the biomass grows over time, i.e. less sunlight can penetrate the underlying water column which reduces the natural NPP production. It is assumed that seaweed plants can be observed by the human eye after 1 month at sea. The growth rate of *Saccharina latissima* is characterized by a sigmoidal curve (Eq. 14 and Figure 24), which is developed based on experimental data from the length of the seaweed blades sampled at different times (March, April, May and even June when harvest was postponed for experimental reasons).

$$Y = \frac{A}{1 + \exp(-(\frac{X - X0}{B}))} \tag{14}$$

Where Y is the radius of the cylinder (m) and X represents the day (X=1 represents the day of deployment, December 15^{th}). In order to fit eq. 14 to the experimental data, the optimal value of the parameters A, B and X₀ have to be found: A=0.74, B=27.58, X₀=128.63 for Ireland and A=0.78, B=19.35, X₀=105.28 for France.



Figure 24 Radius Y of the cylinder over time (from deployment to harvest period); the growth of *Saccharina latissima* is characterized by a sigmoidal curve.

The sea surface occupation related to biomass growth is then calculated as 2 times the radius of the cylinder multiplied by the total length of culture ropes present at sea, i.e. the occupation is modeled as a widening rectangle (two-dimensional top view). Due to the high spore density, the seaweed plants overlap each other during their growth. The natural phenomenon of light scattering together with the dynamic movement of seaweed blades provides a complex light environment with bursts of alternating light and shadow. This complex system is simplified by assuming that the canopy formed by the seaweed blades is dense enough to block light penetration into deeper water layers, i.e. no 'gaps' are assumed within the widening rectangle. At the time of harvest (May 31st), some equipment is removed from the sea (culture ropes, small buoys, etc.). The effective sea surface occupation in the period May 31st – December 15th amounts to 94 m² and 124 m² for Ireland and France, respectively.

The average annual occupation factor α (%) is then calculated according to Eq. 15:

$$\alpha = \left(\sum_{X=1}^{365} \frac{effective sea surface occupation (X)}{total sea surface occupation} x100\right)/365$$
(15)

6.4.3 Results and Discussion

Sea surface occupation factor α

Sea surface occupation of cultivated seaweed, as expressed by factor α (%), was calculated for nearshore seaweed cultivation in Ireland and France throughout the year, taking into account the active growing season and fallow periods preceding and following this time (Figure 25). At the time of deployment, the factor α is 1% (Ireland) and 6% (France) due to the type of equipment used for cultivating the biomass (as shown in Figures 20 and 21). The factor α reaches a maximum value of 9% for Ireland and 88% for France at the time of harvest (end of May), i.e. no full sea surface occupation occurs during the cultivation season. After harvest, site activity is different between the two sites; in France, culture ropes and most buoys are removed from the sea (α factor drops to 2%), whereas in Ireland, the sea surface occupied between the time of harvesting and deployment is negligible (α below 1%). To calculate the life cycle resource footprint of *Saccharina latissima* cultivation at both regions, it is necessary to determine the annual average sea surface occupation factor α , which is 2% and 18% for Ireland and France, respectively. As the model assumed a 20 degree angle and a dense, non-light penetrating canopy, this may lead to an overestimation of the α factor as the complexity of the marine environment is not fully taken into account. More research and experimental data are required on light scattering, turbulent conditions, the way seaweeds hang in the water column and the light permeability of seaweed blades. Despite the fact that the light environment in coastal regions can be greater than suggested in this study, this factor is useful as a first indication of the impact of sea surface occupation and gives insight into the relative difference of both seaweed systems.



Figure 25 Sea surface occupation factor α (%) on a daily basis for nearshore *Saccharina latissima* cultivation in Ireland (longlines) and France (raft system).

Resource footprint of seaweed cultivation

<u>Ireland</u>

The environmental resource footprint of *Saccharina latissima* cultivation in Ireland is presented in Table 12. In total, 1.7 MJ_{ex} of natural resources is extracted to produce 1 MJ_{ex} seaweed biomass, which corresponds to 1997.4 MJ_{ex} day⁻¹ (Appendix C, Table C.5). Due to the long distance between the hatchery and grow-out phase at sea (Appendix C, Figure C.1), the diesel consumption for transport makes a large contribution to the overall

footprint (44.3%). Furthermore, the production of materials (infrastructure) used at the hatchery and sea site requires a considerable amount of raw resources and contribute to 36.6% of the footprint. Especially the production of the culture and anchor ropes is resource demanding (Appendix C, Figure C.7). The environmental resource footprint of seaweed cultivation also considers the occupation of land and sea surface that partially or completely prohibits the production of natural NPP. In Ireland's case, this is translated to a relative contribution of 11.9% (6% due to land occupation and 94% due to sea surface occupation). The direct electricity consumption of machinery has a contribution of 6.8% with the majority of electricity used for lighting the cultivation bottles and tanks in the hatchery (Appendix C, Figure C.9). The impact of fresh water, nutrients and chemicals consumed during the seedling production is less than 1.0%

It can be concluded that fossil resources are mainly consumed during seaweed cultivation (contribution of 75.1%); diesel is produced on the basis of crude oil and power production in Ireland relies mainly on natural gas (54%), hard coal (17%) and peat (9%) (Itten et al., 2012). This has an implication on the use of nuclear resources, which is lower than in countries such as France having a large share in nuclear power. Furthermore, the extraction of marine resources, i.e. sea surface occupation by a human made system, contributes to 11.2% of the overall resource demand. Table C.7 of Appendix C shows more detailed information about the contribution of each flow (material and energy) to the total environmental resource footprint.

	Abiotic	Fossil	Nuclear	Metal	Minerals	Water	Land	Atmospheric	Marine	Total	Contribu-
$MJ_{ex} MJ_{ex}^{-1}$	renewables	fuels	resources	ores			resources	resources	resources		tion (%)
Infrastructure ^(a)	1.9E-02	4.4E-01	7.9E-02	1.3E-03	2.0E-03	7.8E-02	6.4E-03	0.0E+00	0.0E+00	6.3E-01	36.6
Fresh water $^{(b)}$	1.2E-06	9.4E-06	4.4E-06	1.8E-08	1.3E-07	1.5E-04	2.3E-06	0.0E+00	0.0E+00	1.7E-04	0.0
Electricity ^(c)	5.6E-03	1.1E-01	1.9E-03	8.8E-06	2.2E-05	6.8E-03	2.1E-03	0.0E+00	0.0E+00	1.2E-01	7.1
Transport fuel ^(d)	2.1E-03	7.4E-01	8.1E-03	6.1E-05	9.2E-05	8.0E-03	2.7E-03	0.0E+00	0.0E+00	7.6E-01	44.3
Nutrients ^(e)	5.1E-07	3.1E-05	1.3E-06	6.7E-08	5.1E-08	1.2E-06	1.8E-06	0.0E+00	0.0E+00	3.6E-05	0.0
Chemicals ^(f)	4.2E-07	5.4E-06	1.5E-06	7.1E-09	6.6E-08	5.9E-07	2.9E-07	0.0E+00	0.0E+00	8.3E-06	0.0
Surface	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.3E-02	0.0E+00	1.9E-01	2.1E-01	11.9
occupation ^(g)											
Total	2.7E-02	1.3E+00	8.9E-02	1.4E-03	2.2E-03	9.3E-02	2.4E-02	0.0E+00	1.9E-01	<u>1.7E+00</u>	
Contribution (%)	1.6	75.1	5.2	0.1	0.1	5.4	1.4	0.0	11.2		

 Table 12
 Environmental resource footprint of Saccharina latissima cultivation (Ireland), expressed in MJ_{ex} MJ_{ex}⁻¹

^(a) pumps, UV units, compressors, hatchery tanks, glass bottles, ropes, tubes, room chiller, air blower, lighting, autoclave, filters, pipes, anchor blocks, chains, buoys and transport infrastructure; ^(b) to clean the hatchery tanks; ^(c) electricity consumption of TMC UC unit, autoclave, lighting, air blower, room chiller, pumping, compressors, hydrotech drumfilter and Wedeco UV unit; ^(d) diesel consumption of transport by lorry, van and boat; ^(e) NaNO₃ and NaH₂PO₄.H₂O; ^(f) Decon 90 detergent; ^(g) land and sea surface occupation.

<u>France</u>

The cultivation of seaweed in France has a resource footprint of 8.7 $MJ_{ex} MJ_{ex}^{-1}$ (Table 13), which is a factor 5 more than the footprint in Ireland. However, only 512.31 $\mathrm{MJ}_{\mathrm{ex}}$ of resources is extracted per day from nature (Appendix C, Table C.6), which is much lower compared to the case in Ireland because cultivation is at smaller scale at Ventry Harbour. The impact of infrastructure used at the hatchery (CEVA) and for deployment at sea has the biggest contribution (54.7%). Compared to the seaweed facility in Ireland, this could be expected due to material-intensive cultivation system used in France. Figure C.8 of Appendix C shows that the plastic tubes that make the raft system float have a particularly large impact (20.5%) on the resource footprint. This is related to the impact of direct surface occupation (15.6%), of which 84% is due to sea surface occupation and 16% due to land occupation. The production of electricity and use in the hatchery represents 16.2% of the overall footprint. Thus, although electricity is mainly used for only 2 batches of collectors, 5 weeks per year, it is still an important issue. The use of the air blower makes a conspicuous contribution to electricity consumption in the French hatchery (Appendix C, Figure C.10). Furthermore, direct gasoline consumption (approximately 550 L yr⁻¹) used for transport by boat during deployment of the equipment, maintenance and harvest of the biomass contributes to 13.4%. Less transport fuel is used in France compared to Ireland, as the hatchery and sea site are situated much closer to each other (Appendix C, Figure C.1). Similar to the life cycle of seaweed production in Ireland, the impacts of fresh water, nutrients and chemicals are negligible.

A major impact is identified for fossil resources due to the consumption of gasoline and energy during the production of equipment (61.0%). According to the International Energy Agency (IEA) statistics, the electricity production in France relies mainly on nuclear resources (75%), e.g., uranium (Itten et al., 2012). Therefore, electricity-intensive processes such as air blowing consumes nuclear resources, which is translated in a contribution of 17.1% to the total resource footprint. Moreover, the demand for marine resources of raft systems that have a higher average sea surface occupation factor α than a single longline system cannot be ignored (13.1%). More detailed information about the contribution of each flow (material and energy) to the total environmental resource footprint can be found in Appendix C, Table C.8.

	Abiotic	Fossil	Nuclear	Metal	Minerals	Water	Land	Atmospheric	Marine	Total	Contribu-
$MJ_{ex} MJ_{ex}^{-1}$	renewables	fuels	resources	ores			resources	resources	resources		tion (%)
Infrastructure ^(a)	1.0E-01	4.0E+00	3.3E-01	1.5E-02	1.9E-02	1.8E-01	7.2E-02	0.0E+00	0.0E+00	4.8E+00	54.7
Fresh water $^{(b)}$	4.1E-05	3.3E-04	1.6E-04	6.4E-07	4.4E-06	5.3E-03	8.1E-05	0.0E+00	0.0E+00	5.9E-03	0.1
Electricity ^(c)	7.4E-02	1.3E-01	1.1E+00	8.4E-05	1.3E-04	5.3E-02	8.4E-03	0.0E+00	0.0E+00	1.4E+00	16.2
Transport fuel $^{(d)}$	3.9E-03	1.1E+00	1.5E-02	1.0E-04	1.5E-04	1.3E-02	4.5E-03	0.0E+00	0.0E+00	1.2E+00	13.4
Nutrients ^(e)	1.3E-05	7.0E-04	3.5E-05	1.5E-06	1.7E-06	4.2E-05	5.5E-05	0.0E+00	0.0E+00	8.5E-04	0.0
Chemicals ^(f)	3.4E-04	3.1E-03	1.1E-03	8.5E-05	3.4E-05	8.6E-04	2.1E-04	0.0E+00	0.0E+00	5.8E-03	0.1
Surface	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	2.1E-01	0.0E+00	1.1E+00	1.4E+00	15.6
occupation ^(g)											
Total	1.8E-01	5.3E+00	1.5E+00	1.5E-02	1.9E-02	2.5E-01	3.0E-01	0.0E+00	1.1E+00	<u>8.7E+00</u>	
Contribution (%)	2.1	61.0	17.1	0.2	0.2	2.9	3.5	0.0	13.1		

 Table 13
 Environmental resource footprint of Saccharina latissima cultivation (France), expressed in MJ_{ex} MJ_{ex}⁻¹

^(a) hatchery tanks, glass bottles, ropes, tubes, pumps, room chiller, air blower, lighting, autoclave, filters, pipes, anchors, concrete ballast blocks, chains, buoys; ^(b) to clean the hatchery tanks; ^(c) electricity consumption of room chiller, pumping, air blower, lighting and autoclave; ^(d) gasoline consumption of transport by boat; ^(e) NH₄NO₃ and PO₄HNa₂.2H₂O; ^(f) Sodium hypochlorite solution and sodium thiosulfate; ^(g) land and sea surface occupation.

Resource footprint of seaweed compared to microalgae and terrestrial plants

As the competition for terrestrial land is high, especially in Europe, it is interesting to compare the resource footprint of seaweed production in Ireland and France to the cultivation of microalgae and some terrestrial crops with similar moisture content (Figure 26). The study of Taelman et al. (2013) provided the resource footprint (CEENE results) of *Nannochloropsis* sp. production in Belgium at pilot scale (240 m²) and for 2 hypothetical scenarios (1320 m² and 2.5 ha). Life cycle data of the cultivation of sugar beet, maize and potato in Switzerland was taken from the database ecoinvent version 2.2 (Frischknecht and Rebitzer, 2005). Detailed information regarding the resource footprint of these crops are available in Appendix C, Tables C.9- C.13.

Life cycle data are expressed in $MJ_{ex} MJ_{ex}^{-1}$ and are limited to the cultivation and harvest of the biomass, i.e. no further processing steps are considered. For microalgae, cultivation took place in plastic bags and the harvesting stages (microfiltration membrane and centrifuge) were taken into account. After the centrifuge, a concentrate of 18% DM was obtained. For the terrestrial plants, agricultural machinery (e.g., tractors, trailers, etc.) were used to harvest. According to database ecoinvent version 2.2, the DW content of the harvested sugar beet, maize and potato was 25%, 23%, 28% and 22%, respectively.

The total resource demand of seaweed production depends mainly on fossil fuels (especially due to the electricity use of the air blower in the hatchery, the production of infrastructure and the fuel demand for transport). This trend is similar for microalgae production as this biomass can only be cultivated and harvested using energy-intensive processes. The Belgian and French electricity production mix depends more on nuclear resources than the production in Ireland, so more nuclear resources will be extracted to produce the same amount of electricity.

For the terrestrial plants, more than 90% of all required resources are land resources, especially for organically produced crops. Direct arable land occupation for cultivating the biomass and indirect land occupation for the production of manure are the biggest contributors (Appendix C, Tables C.9-C.13). Interestingly, organic production requires more natural resources (especially land) than inorganic production as more green manure

(organically produced) and more direct land is used to achieve the same biomass yield (Appendix C, Tables C.10-C.13).

Seaweed production in Ireland is already quite efficient in terms of natural resource demand compared to the production of terrestrial plants (e.g., 1.7 $MJ_{ex} MJ_{ex}^{-1}$ for seaweed production versus 0.9-3.9 $MJ_{ex} MJ_{ex}^{-1}$ for terrestrial crop production) and is even more efficient than the third (hypothetical) scenario of microalgae cultivation (1.9 $MJ_{ex} MJ_{ex}^{-1}$). Note that a careful interpretation is required as the composition and functionality of the different biomass types are not the same. This could have an effect on the further processing of the biomass, e.g., the higher moisture content of seaweed (10% DM) compared to these terrestrial crops (approx. 24% DM) will require more drying. Therefore, further research into the sustainability of the entire process chain is recommended.

In this study, the biggest potential to improve the footprint of seaweed production is reducing the fuel demand for transport, which contributes to 44% of the total resource footprint, i.e. benefits could be obtained by locating the hatchery and grow-out facility in the same area. In France, at a first trial, the raft system used at sea is subject to friction which results in loss of biomass and an average biomass yield of 5 kg FW m⁻¹ culture rope, which is much lower than in Ireland (25 kg FW m⁻¹). However, at places where there was no friction, a maximum yield of 20 kg m⁻¹ culture rope could already be achieved. Therefore, modifications to the structure of the raft to limit friction are required in order to produce seaweed in a more environmentally sustainable way. At present, attempts are being made to modify the systems to improve the yield.



Figure 26 Environmental resource footprint (expressed in $MJ_{ex} MJ_{ex}^{-1}$) of aquatic and terrestrial biomass production; seaweed from Ireland and France, microalgae from Belgium (S1 = 240m², S2 = 1320m², S3 = 2.5 ha) and sugar beet, maize and potatoes from Switzerland. IP = inorganic production, OP = organic production.

Possible environmental improvements

According to Table C.7 and C.8 of Appendix C, the main bottleneck for seaweed production in Ireland is the fuel demand for transport. In France, the resource footprint is five times as large, mainly because of the lower biomass yield of the system. As the raft structure occupies more sea surface than the single longline system, it is interesting to have a look at the effect of having a larger distance between the culture ropes. Furthermore, the use of plastic tubes at sea is resource demanding, so a scenario with an alternative floating material is analyzed. The aeration device used in the hatchery is also over-sized and thus more efficient equipment could be used.

Improvement Scenario (IS)		Seaweed product	ion (Ireland)	Seaweed production (France)		
		Base case	Improvement	Base case	Improvement	
IS_1	Distance between	335 km	Range of	10 km	/	
	facilities	(hatchery – sea site)	100 km			
		150 km				
		(sea site – DBS ⁽²⁾)				
IS_2	Blower device	0.23 W	/	1.4 kW	0.11 kW	
	(power)					
IS_3	Floating tubes	/	/	HDPE ⁽¹⁾	Softwood	
IS_4	Distance between	Approx. 14 m	/	Approx. 2 m	Approx. 5 m	
	culture ropes					
IS_5	Biomass yield	25 kg FW m ⁻¹	/	5 kg FW m ⁻¹	25 kg FW m ⁻¹	
	-	culture rope		culture rope	culture rope	

Table 14Possible improvements to the life cycle resource footprint of Saccharina latissima
cultivation in Ireland and France.

⁽¹⁾ High density polyethylene

⁽²⁾ Company: Dingle Bay Seaweeds

Table 14 gives a brief overview of possible improvements for the seaweed production systems. In Ireland, the distance between the hatchery (Carna), the sea site (Ventry) and Dingle Bay Seaweeds (Castletownbere) is approx. 490 km. Assuming that 3 hatchery sites at the West coast of Ireland are sufficient to provide seeded culture ropes to all possible nearshore cultivation areas, the distance between a hatchery and cultivation site would be in a range of 100 km (personal communication with Maeve Edwards, NUIG). The impact of reducing transport is analyzed with this scenario. In France, the air blower used in the hatchery during seedling production is over-sized. There are only 2 culture tanks (300 L) in operation at the same time, so a small 55 W blower device per tank at full capacity should provide sufficient aeration (personal communication, Jennifer Champenois, CEVA). Continuous aeration allows a good mixing in the tanks which is important for the seedlings to develop their holdfast and attach strongly to the culture string. Therefore, lowering the operation time of the blower is not recommended.

High density polyethylene (HDPE) is used for the main and intermediate tubes of the raft system. The plastic tubes can be replaced by softwood (e.g., pine), which is also a floating material because it is less dense than water and the tensile strength appears to be higher than HDPE (Bouafif et al., 2009; Ku et al., 2011). The process 'industrial wood, softwood, under bark, u=140%, at forest road' is used from the ecoinvent database. Softwood with low porosity is recommended, otherwise the pores become filled with water and the wood

sinks faster. Because wood may provide a more suitable surface area for settlement of epiphytes, a faster replacement of softwood than HDPE is required (personal communication, J. C.). In this study, half of the life time of the HDPE tubes is assumed for the wooden planks. Moreover, the influence of 5 m distance between the culture ropes instead of 2 m in the original setup in France is investigated. This has an effect on the average sea surface occupation factor α which drops from 18% to 8% with a maximum of 36% at harvest time). Furthermore, the sensitivity of the biomass yield is tested; a scenario with the same average annual yield of 25 kg FW m⁻¹ rope as in Ireland is developed.

In the case of Ireland, limiting the distance between the facilities up to 100 km improves the footprint by 11.4% compared to the footprint of the base case (Figure 27). In the case of France, reducing the power consumption of the air blower reduces the footprint by 17.7% (IS_2). When HDPE tubes are replaced by wooden planks, the original resource footprint drops with 17.9% (IS_3). Most notably, increasing the distance between the culture ropes of the raft system to 5 m increases the life cycle demand for resources during seaweed production by 4%, i.e. despite the fact that the impact of sea surface occupation has been reduced, the demand for more HDPE because of the longer tubes per cultivation unit (50 m instead of 20 m per main tube and 25 m instead of 10 m per intermediate tube) results in a higher footprint than the base case. From Figure 27, it is clear that the environmental impact reduces considerably when productivity increases; the footprint decreases from 8.7 $MJ_{ex}\ MJ_{ex}^{-1}$ to 1.7 $MJ_{ex}\ MJ_{ex}^{-1}$ (comparable footprint as the Ireland base case) which emphasizes the importance of achieving a high yield. Ultimately, it is possible to achieve a life cycle resource footprint of 1.6 $MJ_{ex} MJ_{ex}^{-1}$ (IS_1) and 1.3 $MJ_{ex} MJ_{ex}^{-1}$ (IS_2+3+5) for Ireland and France, respectively, which is comparable to the footprint of the terrestrial plants as discussed in Section 6.4.3 (Figure 27).



Figure 27 Environmental resource footprint (expressed in MJ_{ex} MJ_{ex}⁻¹) of seaweed production (*Saccharina latissima*) in Ireland and France. Results of the base cases as explained in Section 6.4.2 are shown next to 5 improvement scenarios (IS); (IS_1) distance between facilities, (IS_2) power of blower device, (IS_3) floating tubes, (IS_4) distance between culture ropes, (IS_5) biomass yield. IS_2+3+5 represents the resource footprint of 3 improvements.

6.5 Conclusions

This chapter presents a methodology on how to account for marine resources. Those extracted in natural marine systems, such as fish and seaweed, were accounted for by their exergy content (CF) while accounting for sea/ocean occupation of human-made systems was based on the exergy of potential NPP in the upper layers of the ocean. Both temporal and spatial CFs for human-made systems were calculated. An occupation factor α was proposed in case only a part of the euphotic zone was occupied, but more research seems necessary to determine all possible interactions of biological or physical nature. The CFs for natural and human-made systems allowed calculation of the resource footprint of occupying marine areas, which has not been included earlier in LCA. The CFs were implemented in the CEENE method, making it the first LCIA method able to account for marine area occupation. A case study on the macroalgae *Saccharina latissima* production in

Ireland and France is performed and the sea surface occupation factor is determined for both sea sites. This study highlights the usefulness of quantifying the total resource footprint (including marine resources) in a life cycle perspective. Chapter 7

Accounting for land use in life cycle assessment: the value of NPP as a proxy indicator to assess land use impacts on ecosystems⁵

 $^{^5}$ Redrafted from

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7.1 Introduction

Today, a key question in policy, economics and science is: how to manage the use of our available natural resources in a sustainable way? It has become a pressing need to find answers to this question as we are facing global problems such as fossil fuel depletion and fresh water and metal scarcity. One of our most important and scarce natural resources is land, as humans have been using it for thousands of years to fulfill their needs for energy, food and accommodation (Beck et al., 2010). Competition for land among different uses is becoming acute and the pressure of a rising population is taking a huge toll on land quality and affects ecosystem resilience (FAO, 1999). Consequences of an intensified land use include, amongst others, soil degradation and erosion, shifts in ground water availability and loss of biodiversity (Koellner and Scholz, 2007). Therefore, humanity is forced to manage resources more efficiently and environmentally sustainably to limit a long-term environmental damage. In order to ensure land availability for future generations, it is important to reduce the pressure on (fertile) land by e.g., cultivating more aquatic biomas, such as microalgae, on non-arable land instead of terrestrial crops. A critical challenge and essential step involves incorporating a sustainable perspective into land management. As life cycle (impact) assessment (LC(I)A) methods try to evaluate environmental damages due to human activities, they should allow for the evaluation of land use impact on the natural environment. However, accounting for land use impacts in LCA is not straightforward yet due to difficulties in analyzing and modeling the effects on complex natural interactions. Moreover, the need for geographical differentiation in land use impact assessment and the lack of reliable data hinders the application of certain land use indicators in LCA (Teixeira et al., 2016). Consequently, there is still no clear consensus on what kind of land use impacts need to be quantified and, in addition, on the most suitable indicator (Finnveden et al., 2009). Therefore, the present study has three major objectives: (1) identifying the natural land-based processes (e.g., nutrient and water cycling) and its components (e.g. fossil resources) that can be altered due to economic or socio-cultural oriented human activities on land (section 7.2), (2) discussing the possible impact pathways for land use within the LCA framework and reviewing currently existing LCIA indicators that either account for impact on ecosystem health or impact on the amount of natural resources due to land use (section 7.3) and (3), proposing two enhanced proxy indicators (based on NPP) that primarily assess the impact of land use on ecosystem health and providing spatially differentiated CFs for different types of land occupation as development in this field is crucial to improve decision making (section 7.4). These CF's allow to highlight the advantages algae have over terrestrial plants, namely the fact they can be cultivated on marginal land.

7.2 The use of land, consequences for natural land-based processes and components

One of the main challenges in monitoring, modeling and communicating land use impacts is identifying the relation between land cover, land use and the functions of land. Over the past years, land use is often confused with land cover. However, there is a clear difference between the two terms: land cover is defined as the observed (bio)physical cover on the earth's surface (Di Gregorio, 2005), and comprises mainly vegetation and man-made features (incl. water surfaces), while land use most often refers to human activities on land of a certain cover type (Figure 28). Land use is thus a functional dimension. Nevertheless, the relationship between both terms is strong because the dominant land use within a certain area is often related to the existing land cover type. The anthropogenic land use activities can include biotic (e.g., clearcutting of tropical rainforest) and abiotic resource extraction (e.g., mining), surface use for biotic production (e.g., agriculture) and non-biotic matters (e.g., housing, recreation). Land use change, often linked to these human activities, refers to the change from one land use type to another, which regularly leads to a change in land cover (Matilla et al., 2012). When there has been no land use (or it happened a considerable time ago) at a given location, the present land cover corresponds to the natural vegetation (e.g., natural forest). In contrast, land use by humans at present or in the recent past generally results in a land cover that is not natural for that specific location (Koellner et al., 2013a).



Figure 28 Definition of land terms; land cover, land use, land occupation and land transformation or land use change (based on Goel, 2013).

7.2.1 Land functions as defined in literature

To encourage more sustainable land-management practices, it is important to first consider the functions of land. Several attempts have been made in recent studies to identify different types of land functions that are associated with natural, semi-natural and man-made ecosystems. De Groot et al. (2002) defined ecosystem functions (EFs) as the capacity of natural processes and components to provide goods and services that satisfy humans, directly or indirectly. In total, 23 ecosystem functions were described and grouped in four primary categories: regulation, habitat, production and information (Table D.1 of Appendix D). Each function is a result of a natural process and it provides goods and/or services that are valued by humans. Only those goods and services that can be used on a sustainable basis are included, i.e. non-renewable natural resources such as oil and gold are excluded because extracting these resources could impair the integrity and proper functioning of certain ecosystem processes. In 2005, the Millennium Ecosystem Assessment (MEA) report provided a conceptual framework that can be used in land management. It determines the ecosystem goods and services (also referred to as our natural capital), defined as the benefits people obtain from ecosystems (Table D.1). Goods are material products resulting from ecosystem processes, such as fossil fuels, wood, minerals and fiber. Ecosystem services were grouped according to their value for humans into four main categories: 1) provisioning, such as the production of food and water; 2) regulating, such as the control of climate and erosion; 3) supporting, e.g., nutrient cycling and soil formation; and 4) cultural, such as ecotourism and spiritual and recreational values (Millennium Ecosystem Assessment, 2005). These categories roughly correspond to the regulation, habitat, production and information categories as defined by de Groot et al.

(2002) (de Groot and Hein, 2007). According to MEA (2005), humans have substantially altered all 31 goods and services by exceeding the capabilities of ecosystems to provide the services.

It is clear that an anthropocentric perspective forms the basis of both concepts (Silva, 2011). Ecosystem functions and ecosystem goods and services were mainly focused on ecosystem processes and natural components (the environmental pillar of sustainability) in light of the benefits they can provide to human well-being. Both concepts were not considered to be sufficiently comprehensive to include the requirements for a full sustainability analysis of land use impacts (Silva, 2011). Therefore, within the SENSOR project, a land use functions (LUFs) framework was developed, based on the concepts of multifunctionality, EFs (de Groot et al., 2002) and ecosystem goods and services (Millennium Ecosystem Assessment, 2005) but now addressing the most relevant economic, environmental and societal functions of land, representing the three conventional pillars of sustainability (Pérez-Soba et al., 2008). These LUFs were defined as 'the private and public goods and services provided by the different land uses that summarize the most relevant economic, environmental and social aspects of a region'. In total, nine single LUFs were identified: provision of work, human health and recreation, cultural (mainly social use functions), transport, land-based production, residential and land independent production (mainly economic use functions), provision of abiotic resources, support and provision of biotic resources and maintenance of ecosystem processes (mainly environmental use functions) (Table D.1) (Pérez-Soba et al., 2008). Regulating ecosystem processes and support of biodiversity were considered as the provisioning land use functions, which have undeniably primarily an environmental function, i.e. implying a function towards the natural environment. However, provisioning functions can be interpreted differently as they fit also in an anthropogenic perspective, i.e. implying a function towards mankind (Dewulf et al., 2015). More information about these approaches (EFs, ecosystem goods and services and LUFs) can be found in Table D.1.

A broader and more flexible term 'land function (LF)' was thereafter introduced to comprise all possible interpretations of ecosystem goods and services, EFs and LUFs, as it referred to the capacity of land use systems and ecosystems to provide goods and services

within the landscape (Silva, 2011; Verburg et al., 2009). It did not only include the goods and services related to the intended land use (e.g., production of corn) but also the goods and services (e.g., esthetic natural beauty) that are often unintended by the owner of the land. However, apart from a clear definition of LFs, an exhaustive list of these LFs is missing.

7.2.2 Identification of natural land-based processes and resources

It is worth to critically have a look at previously discussed approaches that address the functions of land because they are not directly suited to fit in the conventional LCA framework where particular attention is paid to the depletion of natural resources and damage to ecosystem processes. Therefore, in this study, one of the objectives is to identify the natural land-related processes (e.g., primary production and erosion regulation) and natural land-related resources (stocks and funds) as a starting point to assess land use impacts. Stocks or deposits are classified as unrenewable as they are not regenerated within human lifetimes (e.g., fossil fuels, sediments), while funds are renewable (e.g., biomass and groundwater) (Dewulf et al., 2015). Based on the production EFs of de Groot et al. (2002), the provisioning ecosystem goods of the MEA report (2005) and the work of Dewulf et al. (2015) where natural resources were inventoried according to their origin, 8 land-related natural resources can be distinguished: biomass, sediments, fossils, metals, minerals, (fresh) water, genetic resources and land surface (Figure 29). Furthermore, the natural land-related processes include the regulating and supporting ecosystem processes as described in the MEA report (MEA, 2005), in addition to the support of biodiversity (Figure 29) (Pérez-Soba et al., 2008). Some of these processes are strongly linked to the availability of natural resources, e.g., the primary production process supports the autotroph biomass standing stock. Human activities on land affect the amount of these natural resource assets and/or disturb certain ecosystem processes. Based on the MEA report and the LUFs framework, the driving forces behind land occupation and transformation could be identified as being primarily socio-culturally (e.g., land used for recreation) or economically (e.g., mining) oriented, as shown in Figure 29 (MEA, 2005; Pérez-Soba et al., 2008). In fact, all natural land-related processes and the availability of natural resources have (to some extent) a socio-cultural value because they (indirectly)

contribute to human health and well-being (clean air, production of food, heating, etc.) and the provisioning of cultural aspects. From an economic point of view, all natural resources (incl. land surface) are highly relevant, e.g., for the production of medicines, the supply of potable water and food, infrastructure, transport and machinery (Dewulf et al., 2015). The economic benefits obtained by converting these land resources into products for human welfare may jeopardize ecosystem functioning and the ability to provide ecosystem services. Therefore, one should strike a balance between the preservation of our natural ecosystems, as such, and the (mis)use of all goods and services to improve the overall well-being at a community or individual level (Schaubroeck et al., 2015). In trying to find a proper balance, it is important to estimate the overall damage land use can possibly have on the environment, both on natural resources and processes.



Figure 29 Anthropogenic land use, which is mainly socio-culturally or economically oriented as defined in MEA (2005) and Pérez-Soba et al., (2008), affects the amount of natural stocks and funds (e.g., fossil resources) and/or disturbs certain ecosystem processes (the maintenance of biodiversity, supporting or regulating processes) (de Groot et al., 2002; Dewulf et al., 2015). The driving forces behind land use are shown in the left bloc of the figure and the natural land-based resources and ecosystem processes that may be affected by land use are identified (upper and lower right blocs, respectively).

7.3 Assessing the impact of land use

Recognizing the central role of our natural capital for sustainability, there is a need to assess (potential) land use impacts in a quantitative way. The most used framework is a comprehensive state-of-the-art LCA, a tool often used to support decision making (Koellner et al., 2013b). However, conventional LCA is a methodology developed to assess mainly potential environmental impacts. To analyze the social and economic impacts of land use, there is still a need for consistent and robust indicators and methods within the frameworks of social LCA (SLCA) and life cycle costing (LCC) (Finkbeiner et al., 2010). This study concentrates primarily on the environmental impacts of land use on ecosystem processes and natural resources. First, an LCA framework for terrestrial land use impact assessment is discussed where possible impact pathways linking human land interventions to impact categories are identified. Second, a brief review of existing and operational land use impact indicators is performed.

7.3.1 An analytical framework to assess the environmental impact of land use in LCA

A conventional LCA is carried out according to 4 main phases, as described in the ISO 14040 and 14044 guidelines. More information can be found in section 2.1.

In order to assess land use impacts in LCA, data regarding the previous land cover/use type (mostly a reference situation, e.g., the land type in the absence of any human intervention), the current land cover/use type, the time and area of land use and the biogeographic location (Milà i Canals et al., 2014) should be collected during the LCI step (Figure 30). In LCA terminology, land use is often referred to as land occupation and land use change as land transformation (both elementary flows), expressed in m^2 yr⁻¹ and m^2 , respectively (Lindeijer et al., 2002; Matilla et al., 2012; Milà i Canals et al., 2007_b).



Figure 30 Key elements currently employed on the assessment of land use and land use change impacts in LCIA (based on Milà i Canals et al., 2014)

For the impact assessment step, elementary flows are linked to (multiple) impact categories (such as land occupation) and the impact can be quantified by multiplying the amount of each elementary flow with its respective CFs, which are values that express the impact per elementary flow amount. Often, site-generic CFs, implying no distinction to where resources come from or emissions go to, are used for the background system. For the system under study (foreground), it is recommended to calculate spatially differentiated CFs to include site-dependent impacts of land use, i.e. CFs per region and per land use type are desired in specific LCA studies (Koellner et al., 2013b; Milà i Canals et al., 2007b; Saad et al., 2011). The CFs can be defined at midpoint (problem-oriented approach where impacts are translated into environmental themes such as acidification) or endpoint level (damage-oriented approach where impacts are translated into damage to issues of concern), as shown in Figure 30. Endpoints are easier to understand for decision-making purposes but the uncertainty is higher compared to midpoint results due to the complex modeling behind it (Souza et al., 2015). The issues of concern are the safeguard subjects or areas of protection (AoP) we like to protect or sustain and it helps us to define the impacts that should be assessed and modeled (Dewulf et al., 2015). Traditionally, the AoPs as considered in conventional LCA are human health, natural resources and natural environment or ecosystem health (Dewulf et al., 2015; European Commission, 2011).

A guideline on a comprehensive and consistent impact assessment of land use is proposed by Koellner et al. (2013b) and is based on the 'Key elements in a Framework for Land Use Impact Assessment Within LCA' paper that was developed in the context of the UNEP-SETAC Life Cycle Initiative (Milà i Canals, 2007b). The land-use framework consists of a linear area-time model that combines spatial and temporal dimensions. Land occupation and transformation have an impact on the ecosystem quality (Q), which can be measured with different indicators expressing e.g., depletion of metals, the biodiversity potential (species richness) or the value of ecosystem processes (e.g., primary production and water regulation), expressing impacts at midpoint or endpoint level. More detailed information about this framework is available in Figure D.1. Whereas this framework is currently the most feasible one to assess potential environmental impacts of land use on ecosystem quality (Q), there is still no clear consensus on how this Q should actually be quantified, i.e. which types of land use impact exactly have to be assessed (Achten et al., 2008; Finnveden et al., 2009).

In an attempt to clarify the main impact pathways related to the use of land (occupation and/or transformation), a cause-effect chain is developed as part of the second objective of this study (illustrated in Figure 31). According to Koellner et al. (2013b) and Souza et al. (2015), human interventions on terrestrial land can include the spread of chemical substances on land, irrigation and drainage, soil compaction and fragmentation, surface sealing, vegetation cover modification and (over)exploitation of resources. These interventions lead to direct impacts on natural stocks and funds and/or ecosystem processes. At midpoint level, the loss of natural resources or loss of certain ecological processes or biodiversity due to land use can be assessed by several indicators. These impacts can result in a damage to the AoP natural resources, the AoP natural environment and/or the AoP human health (endpoint level), the AoPs as defined in classical LCA, because all ecosystem processes and the production and availability of land resources are strongly interlinked (Figure 31). For example, if human activities on land disturb the natural water cycling process, it can affect biomass and fresh water availability which may cause damage to all AoPs.



Figure 31 Cause-effect chain of land occupation and transformation; human interventions on land affect the amount of natural stocks and funds and/or disturb certain ecosystem processes (midpoint level) which in turn leads to a damage to the AoP natural resources, AoP ecosystem health, and/or AoP human health (endpoint level).

7.3.2 LCIA indicators that assess the environmental impact of land use

Lately, several indicators are developed to quantify land use impacts within the LCA framework. This paper reviews a set of LCIA indicators that account for land use-related environmental impacts, i.e. indicators that assess a change in the AoP natural resources or the AoP ecosystem health. The AoP human health goes beyond 'environmental' as it is clearly an anthropocentric interpretation of the environment (Dewulf et al., 2015). In fact, this AoP was included in conventional LCA before the development of social LCA (Paragahawewa et al., 2009).

Given the complexity of biodiversity, ecosystem functioning and its interactions, all the different potential environmental impacts cannot be understood by applying one single indicator because nowadays these typically cover only a limited set of land use impacts (Dewulf et al., 2015; Goel, 2013; Matilla et al., 2012). This implies, for instance, that the choice of indicators will depend on whether the priority is to assess a disturbance of specific ecosystem processes or a depletion of natural land-based stocks and funds. Note however that these aspects can be strongly interlinked (see also Figure 31). As a result, some indicators act as a proxy to determine the impact on the AoP natural resources or AoP ecosystem health: e.g., 1) primary production losses can be a proxy for a reduced amount of natural biomass (AoP natural resources) and 2) declining genetic resources can be a proxy to assess the impact of land use on biodiversity (AoP ecosystem health). In this section, only the most commonly applicable land impact indicators that fit in the LCA framework, i.e. where CFs are identified or proposed, are discussed (Figure 32).


The SED indicator of Rugani et al. (2011) and the Ecological footprint method as proposed by Venetoulis and Talberth (2008) and Huijbregts et al. (2007) are difficult to classify in the AoP natural resources or AoP ecosystem health. * The soil quality indicator as developed by Milà i Canals et al. (2007a) and Brandão et al. (2011) determines the impact land use has on certain life support functions (ecosystem processes) such as biotic production (NPP), substance cycling and buffer capacity and climate regulation.

Figure 32 Classification of LCIA indicators (state-of-the-art, mentioned as a reference to the authors' work) that are originally considered to assess the impact of land use on the AoP natural resources or the AoP ecosystem health. The proxy used by each indicator is mentioned. Only the LCIA indicators where CFs are identified or proposed, are presented.

Impact of land use on the AoP natural resources

Land supports and provides biotic (biomass and genetic resources) and abiotic (sediments, fresh water, fossils, metals, minerals) resources (Koellner, 2000; Swart et al., 2015). As (some of) these natural resources are degrading rapidly, it has become an area of concern (AoP natural resources). The risks of natural resource depletion induced the development of LCA indicators that were able to quantify the impact land use (potentially) has on the amount of natural stocks (unrenewable, e.g., metals) and funds (renewable, e.g., biomass),

as these are mainly affected in a negative way (Milà i Canals et al., 2014; Millennium Ecosystem Assessment, 2005). In this section, LCA indicators that either assess a change in biotic resources or in abiotic resources are discussed.

LCIA methods that assess the impact of land occupation and/or transformation based on a change in *biotic resources* can be divided in methods accounting for an alteration in biomass or genetic resources. For net biomass yield as such, different from net primary production, methods are lacking. However, NPP, the net amount of carbon (biomass) assimilated through photosynthesis in a given period by vegetation, can be used as a proxy to assess a change in biomass resources (see also Figure 29). Haberl et al. (2007) developed the 'human appropriation of net primary production' HANPP indicator that assesses the intensity of land use. This indicator measures the difference in the free NPP left for ecosystems between the current land vegetation, i.e. after human intervention where NPP loss due to harvested biomass and change of land functions is taken into account, and a reference natural situation. The latter has been described as the NPP of the potential natural vegetation (PNV) which is the natural state of vegetation without human intervention (Haberl et al., 2007; Zhang et al., 2010). However, at that point, despite the strong theoretical background, the HANPP indicator as developed by Haberl et al. (2007) was not directly applicable in LCA as there were no (site-specific) CFs calculated (Matilla et al., 2012).

A thermodynamically-based resource accounting method that accounts for the impact of land use is the CEENE method, of which to date three versions exist: CEENE 2007 (Dewulf et al., 2007), CEENE 2013 (Alvarenga et al., 2013) and CEENE 2014 (Taelman et al., 2014). In the initial version, the exergy content of the solar radiation that can be metabolized through photosynthesis by natural ecosystems (set at 2% of the total irradiation) was quantified to be 68.14 MJ_{ex} m⁻² yr⁻¹ for Western European conditions. This means that land occupation within an industrial system (e.g. intensive agriculture) deprives the natural ecosystem from solar exergy, per unit area and time, and this is used as a proxy for impact of land occupation on natural biotic resources. As this approach did not take into account other (local) factors such as climate and land quality, the exergy content of the potential natural NPP was afterwards introduced as a better proxy for the value of land and spatially

differentiated CFs at grid, region, country and continent level were calculated in CEENE 2013 (Alvarenga et al., 2013). The site-specific CFs are expressed in exergy terms as it allows easy comparison with other (midpoint) impact results because all sorts of resources can be expressed in their respective exergy content. The framework of Alvarenga et al. (2013) is based on a distinction between natural and man-made systems, whereby in natural systems one should account for the (exergy content of the) harvested biomass and in man-made systems for the loss of natural potential NPP because the extracted biomass is not a natural resource in the sense that this resource would not be available in the absence of human intervention (land is the original resource). Therefore, the CFs where calculated based on a [0] (natural systems) - [1] (man-made systems) accounting approach: CFs for land occupation in a natural system were set to zero (only accounting for the extracted biomass) and the CFs for land use in, for example, European man-made systems were equal to 23.20 MJ_{ex} m⁻² yr⁻¹, which represents the average natural potential NPP (NPP₀) in Europe, regardless of the type of land that was occupied. The same approach was used to account for marine resources (biomass and sea surface occupation), which resulted in the extended CEENE 2014 version (Taelman et al., 2014).

In addition to the CEENE method, a spatially-differentiated LCIA indicator on land use impacts based on the HANPP approach was developed by Alvarenga et al. (2015). This is a 0 to 1 or [0-1] interval accounting approach because the actual NPP loss of anthropogenic land use is accounted for. CFs were calculated at midpoint level for 162 countries and 4 land occupation types (cropland, pasture, infrastructure and wilderness) as proposed by Erb et al. (2007), and expressed in kg DM m⁻² year⁻¹.

The study of Brandão and Milà i Canals (2013) showed that soil organic carbon (SOC) can be used as an indicator to assess impacts on the biotic production potential (BPP) (AoP natural resources) of ecosystems, referring to a change in the productive capacity or the ability of the ecosystem to sustain future biomass production. BPP depends to a large extent to aspects such as climate, vegetation cover and soil type, aspects that determine soil quality. Spatially differentiated CFs for land occupation and transformation (kg C m^{-2} yr⁻¹) are developed for a variety of land uses and climate regions for BPP.

On the other hand, land use can trigger changes in the amount of genetic resources, categorized under the AoP natural resources. However, LCA indicators use a change in genetic resources as a proxy to determine the impact of land occupation on biodiversity, categorized under the AoP ecosystem health. As a result, these indicators are discussed in the next section.

Land use can also lead to *abiotic resource* depletion. In this context, Núñez et al. (2013) developed a globally applicable and spatially resolved method for assessing the impact of land use on the loss of topsoil reserves (AoP natural resources) due to soil erosion. To quantify the effect of erosion by land uses, the amount of emergy (solar energy) was calculated of which the natural system is deprived of to yield the new stock of soil. The CFs are based on the difference in soil depth (FAO's soil depth classification map), and an emergy factor of 24 MJ solar energy per gram of soil (used for all types of soil and locations). Spatially explicit CFs on a grid cell-level resolution (approximately 10×10 km²) for the entire world are provided. It is however questionable to express a soil amount in terms of solar energy.

Apart from this indicator, there is a lack of LCIA indicators that link a change in the amount of non-renewable abiotic resources with land use impacts within the AoP natural resources. However, many indicators do account for the impact of extracting abiotic resources but do not relate this to land use impacts as they assign this impact to a separate impact category other than land use (e.g., the impact category 'mineral resource depletion' of the ReCiPe method of Goedkoop et al. (2009) and the the category 'metal ores' of CEENE method of Dewulf et al. (2007) and Alvarenga et al. (2013)). As stated by many authors, land use affects the provisioning of many abiotic resources, however, this has not yet led to the development of a wide range of indicators that assess the impact on abiotic natural resources of as a primary cause of land use (Rørbech et al., 2014; Swart et al., 2015). Therefore, further research on this matter is needed.

Impact of land use on the AoP ecosystem health

Land use can affect certain regulation or supporting ecosystem processes in a positive or negative way (Figure 29) (Saad et al., 2013). In this section, LCA indicators that assess land use impacts on the basis of altered supporting and/or regulating ecosystem processes, primarily referring to the AoP ecosystem health, are discussed.

As explained in the first part of section 7.3.2, land use can affect *biodiversity*, which can refer to genetic, ecosystem or species variation (number of species) (Weidema and Lindeijer, 2001). Genetic diversity forms the basis of a possible diversity at the higher levels. However, most indicators are developed to model land use-related species diversity impacts in LCA as data is most easily available at this level. Biodiversity loss is often quantified as a direct measurement of species richness, mostly based on the Species-Area Relationship (SAR) of Sarkar and Margules (2002), i.e. the larger an area of land is, the greater variety of species it will contain, a relation that is poorly understood (Souza et al., 2015). As a result, these indicators use the SAR as a proxy to determine the impact of land use on biodiversity and in the broader sense on ecosystem health.

The most comprehensive developed and globally applicable LCA indicators that account for biodiversity loss due to land use are now discussed. A first approach to assess land use impacts on biodiversity for certain biomes was developed by Weidema and Lindeijer (2001). The method is based on vascular plant species richness, inherent ecosystem scarcity and ecosystem vulnerability but, due to the lack of empirical data, model and data uncertainty may be considered high (de Baan et al., 2013a).

The Ecosystem Damage Potential (EDP), developed by Koellner and Scholz (2007), is an indicator which relies solely on species richness of vascular plants. This may be a proxy for the richness of other species as well (Michelsen and Lindner, 2015). CFs were calculated for a series of land use types and intensity classes to determine the endpoint oriented damage potential (Koellner and Scholz, 2008). It is recommended to only use the CFs for land use in Central Europe. Compared to EDP, the Biodiversity Damage Potential (BDP) is based on relative changes in species composition to assess land use impacts across numerous land use types, world regions and also several taxonomic groups (De Baan et al., 2013a). The

same concept of BDP is used for the Swiss Ecological Scarcity model in which the loss of biodiversity is measured for the differentiated biomes and scientifically classified land use types (Frischknecht and Büsser Knöpfel, 2013; Frischknecht et al., 2006).

Likewise, Ecoindicator 99 provided a scale expressing species diversity per type of land use, depending on both the size of the area and the type of land use. Two sub-categories for land use could be distinguished; land occupation and transformation wherein for each case local as well as regional effects were considered, expressed in Potentially Disappeared Fraction of species (PDF) (m² yr) (Goedkoop and Spriensma, 2001). In contrast to the previous endpoint indicators, the CFs of the IMPACT 2002+ method determine the rate of species loss and were directly taken from Eco-indicator 99, moreover excluding potential impacts from land transformation (Jolliet et al., 2003). Also Recipe 2008 was derived from Ecoindicator 99, reflecting the damage to ecosystems due to both the effects of occupation and transformation of land (Goedkoop et al., 2009). Analogue to Ecoindicator 99, these indicators express the impact of both land use types in PDF x yr at endpoint level.

Vogtländer et al. (2004) expanded the traditional modelling of biodiversity to a combination of on one hand the number of species of vascular plants and on the other hand the rarity of ecosystems and their plants. However, in most of the cases, the respective species richness model seemed to be sufficiently accurate to model biodiversity. A limitation of this work is that the methodology is mainly developed to assess biodiversity loss in the Netherlands and no CFs are available to be used at a global scale. In contrast to the methods relying on plant species, the method introduced by Geyer et al. (2010) evaluated spatially explicit land use impacts based on a habitat suitability matrix relating terrestrial vertebrate species native to habitat types but the underlying input data were however only available for California. A more general approach was proposed by De Baan et al. (2013_b) in which CFs concerning species richness for five species groups (mammals, birds, amphibians, reptiles, and plants) were calculated. To overcome the challenges in previous methods, improvements were made in terms of spatial variation and level of threat of each species and rarity across the world by De Baan et al. (2015). Their method based on habitat suitability models of mammal species.

Apart from quantifying biodiversity loss as a direct measurement of species richness, the consideration of other aspects of biodiversity such as variability, quantity, functionality and distribution of species are important and are often absent in the current land-use modeling activities due to the complexity of the interactions between species among each other and their habitats. A first method to account for functional aspects of biodiversity (e.g., feeding behavior of animals) is proposed by Souza et al. (2013). This indicator can determine the role species fulfill in their communities and how they influence the way ecosystems operate. Significant differences exist between CFs for species richness and functional diversity for different taxonomic groups (mammals, birds and plants) and across land use types. It is recommended to use the CFs of functional diversity as a complement to current practice. Given the difficulty in grasping the value/functionality of biodiversity to mankind and the issues in assessing all its aspects, further research is imperative.

Additionally, land use can have an effect on *water flows*. Maes et al. (2009) developed an indicator to assess the impact on blue water (water resources immediately available for mankind, e.g., surface water) and green water (water vapor due to evapotranspiration) flows (water cycling processes). The evapotranspiration rate of the PNV was used as a reference. As only a model was proposed, there were no global or site-specific CFs calculated that can be used in an LCA study, except for some land use types in Belgium.

In order to address *soil quality* impacts, Milà i Canals et al. (2007a) and Brandão et al. (2011) developed a SOC indicator, expressed in kg C m^{-2} yr⁻¹, which is linked to soil physical, chemical and biological fertility. However, this indicator is only developed for agricultural and forestry systems, i.e. there are no CFs for other types of land uses as defined in many LCA databases such as ecoinvent (Frischknecht et al., 2007). Furthermore, the indicator does not account for other soil ecological functions such as the regulation of water, air and erosion.

Saad et al. (2011) developed a comprehensive assessment method that evaluates the impact of land occupation on 3 *regulation processes*; erosion regulation potential (ERP), fresh water regulation potential (FWRP) and water purification potential (WPP). This method is based on the LANCA tool (Land Use Indicator Value Calculation in Life Cycle Assessment) that predicts the impacts of land occupation on:

- erosion resistance
 (related to ERP, expressed in tonnes of soil eroded per hectare per year)
- mechanical filtration
 (related to WPP, expressed as the rate of water passing a given time unit)
- physicochemical filtration
 (related to WPP, expressed in centimole of cation fixed per kg of soil)
- groundwater replenishment

(related to FWRP, measured as the amount of water recharged during a year) Detailed information is available in the work of Beck et al. (2010). Spatially differentiated CFs were calculated per land use type for Canadian ecozones and ecoregions. By Saad et al. (2013), the same approach was used to provide spatially differentiated CFs for a worldwide scale. Land transformation interventions were assessed based on rough estimates of regeneration times as a function of latitude and altitude (van Dobben et al. 1998).

As described in section 7.3.2 (under part AoP natural resources), Núñez et al. (2013) developed CFs to express soil loss as damage to the AoP natural resources. In addition, land occupation leads to *soil erosion* and altered soil functions, which affects NPP and leads to damage to ecosystem quality, i.e. NPP loss was selected as a proxy indicator for damage to the AoP ecosystem health. Spatially-differentiated CFs were calculated to express NPP losses based on linear relationships between NPP and SOC losses.

Land use can also lead to an impact on *climate change* by influencing the carbon sequestration in the top soil and land cover. Within the framework of the UNEP/SETAC Life Cycle Initiative, the study of Müller-Wenk and Brandão (2010) developed a Carbon Sequestration Potential (CSP) indicator that calculates emission factors as a result of changes in carbon storage in vegetation and soil due to land occupation and transformation. The carbon quantities are expressed as 'fossil-combustion-equivalent' tonnes of carbon per hectare transferred to air, which makes it possible to assess global warming potential in LCA.

There are two indicators that are difficult to classify between the AoP natural resources and the AoP ecosystem health: the Solar Energy Demand (SED) method and the Ecological Footprint.

The SED method of Rugani et al. (2011) used a system boundary that differed from all other indicators discussed in this paper; the boundary was located between the ecosphere (geobiosphere) and the external environment instead of between the natural environment and the technosphere (human-industrial system) (Schaubroeck et al., 2013). This indicator expressed the amount of external energy (mainly solar but also tidal and geothermal) in amounts of emergy (solar energy equivalents) needed to produce a good or service and calculated emergy factors for different natural resources, including land. This implies that through the emergy framework one accounts for the contribution of local and free resources (e.g., rain, sun, wind) that feed a certain area (in m²) over a year, i.e. to account for the energy required to obtain a resource. Instead, land resources were characterized by one single factor, 6.17 x 10^4 MJ_{se} m⁻² year⁻¹, which is the average value of the empower density of the Earth (9.26 x 10^{18} MJ_{se} year⁻¹, Odum, 1996) divided by the total land area (1.50 x 10¹⁴ m²). Thus, while the other indicators tried to account for the intrinsic value of the resources, SED attempted to characterize the preceding effort (in terms of solar energy flows) spent by the ecosphere in generating resources. Therefore, it might be questioned as an appropriate indicator for the purpose of assessing the consequential impact of land use. Furthermore, no specific regionalized solar energy factors were provided (Huysveld et al., 2015; Liao et al., 2012).

The Ecological Footprint is defined as the amount of biologically productive land and surface water area needed to regenerate the resources that are consumed by human population and to absorb part of the waste generated by fossil (carbon dioxide emissions) and nuclear fuel consumption (Wackernagel and Rees, 1996). It has a strong anthropogenic basis. Here productivity originally refers to the potential to achieve maximum agricultural production at a specific level of inputs (e.g., fertilizer). It differs from measures of ecosystem productivity such as NPP, which encompass all biomass, including undergrowth, bark, leaves and sub-soil plant parts (Wackernagel et al., 2005). A more advanced version of the ecological footprint was developed by Venetoulis and Talberth

(2008). One of the modifications included changing the basis of calculating equivalence factors for each type of land from the potential of land to provide food for humans to the total NPP of various ecosystem/biomes. However, potential NPP data could be used as weighting factors. Huijbregts et al. (2007) made the ecological footprint methodology applicable to assess environmental burdens by a wide range of products consumed in the western economy. However, the proposed CFs to be used in LCA were based on the original version of the ecological footprint. The ecological footprint covers both land occupation impacts and carbon dioxide emission, but does not include classical LCA midpoint or endpoint categories (European Commission, 2010a), which makes it more difficult to classify this method under the AoP natural resources or ecosystem health.

7.4 Advanced NPP-based LCIA indicators

Based on the review of the LCIA indicators that assess certain impacts of land use (section 7.3.2), it became clear that NPP is a key process for life on earth. Most indicators use (potential) NPP as a proxy to assess changes in the amount of natural land-related stocks and/or funds (AoP natural resources). However, in this study, NPP is proposed to be a good starting point for determining the possible impact land use can have on the AoP ecosystem health (Langlois et al., 2014).

7.4.1 NPP as a key indicator for ecosystem functioning

NPP (usually expressed as g carbon m⁻² year⁻¹) equals the gross amount of biomass produced by autotrophic organisms through photosynthesis minus the amount that is respired by themselves, i.e. NPP is defined as the net amount of carbon assimilated in a given period by vegetation. This vegetation (the autotrophs) form the base of the entire food web, both on land and in the oceans. NPP is a key measurement of the storage of chemical energy that is available to consumers in the ecosystem, which is controlled by physical, environmental and biotic factors. Thus, NPP is closely related to the resilience of ecosystems, the buffering capacity and absorption ability of wastes and emissions, and to the supply of products and services to humans (Erb et al. 2009). Damage to a key

supporting process such as NPP, as defined by MEA (2005), can have a tremendous impact on the earth's environment because many aspects of ecosystem functioning such as nutrient cycling, the concentration of atmospheric dust, the hydrological cycle and buildup of organic material depend on the amount of trophic energy available for transfer from plants to other levels in the ecosystem web. In addition, there are indications that NPP is positively correlated with biodiversity (Costanza et al., 2007; Pfister et al., 2009). A disturbance of this process may have tremendous impact on the functioning and health of ecosystems, and eventually on life on earth. Therefore, it is often stated that (changes in) NPP is a good proxy midpoint-indicator for the impact on supporting and regulating ecosystem processes (Beck et al., 2010; Goedkoop et al., 2009; Langlois et al., 2014; Weidema and Lindeijer, 2001).

However, based on the review, NPP can also be used as a proxy to determine biomass loss due to land use, e.g., as was done in the CEENE (2013) method. Certainly, NPP is an ecosystem process that has a strong linkage with the standing biomass stock (Figure 31). However, NPP differs from standing biomass stock because the carbon release by heterotrophic respiration (which is presumably difficult to estimate on a global scale) is not taken into account (Allan and Kling, 2006; Kirschbaum et al., 2001). For example, Nagy et al. (2006) estimated that for a managed forest about 80% of the NPP was heterotrophically respired (mainly decomposition of soil organic matter) and only 20% ended up as real biomass increment (available as stock for harvest). A large share of the NPP after all can end up in the soil (leaves/needles, fruits) and is consumed by microbiota, this especially in natural systems. Mankind can only to a certain extent prevent this heterotrophic respiration by harvesting prior to consumption and decomposition of NPP, however, then the system can most probably not be considered natural anymore. Moreover, too much harvest leads possibly to nutrient depletion (Vangansbeke et al., 2015), and thus ecosystem malfunctioning. Due to this critical remark, it was concluded that NPP serves as a very relevant proxy for determining land use impacts on ecosystem health.

7.4.2 State-of-the-art NPP-based LCA indicators

As discussed in section 7.3.2, there are three key NPP-based indicators developed by Alvarenga et al. (2013, 2015) and Núñez et al. (2013) to assess certain environmental impacts caused by anthropogenic land use in LCA. Table 15 summarizes the innovative aspects and limitations of these land indicators. The indicator developed by Alvarenga et al. (2013) provided no land use type specific CFs. Thus, despite the fact that the CEENE method accounts for the loss of natural resources (before any human intervention), the intensity of land use was not considered and no natural value was assigned to the remaining NPP after land use. However, it neither seems aphoristic nor fair to account for land use impacts on ecosystem processes due to e.g., urban settlement in the same way as for an intensive forest. In many LCIA methods, a line is drawn very strictly among types of systems between human-made and natural ones while in fact certain land types are not strict human-made or natural. From a resource perspective, the semi-natural NPP is still a valuable resource for many ecosystems and the natural environment as a whole, which can be of benefit to human society (Pérez-Soba et al., 2008). A few years later Alvarenga et al. (2015) introduced another NPP-based indicator. The HANPP approach was used to calculate the CFs for 4 types of land uses, though an insufficient amount to be compatible with product datasets available in process-based LCA databases. Furthermore, because these CFs are not expressed in exergy terms, a direct comparison with other resourcerelated impact categories could be hampered. Alvarenga et al. (2015) pointed out that this should be a next step. Both indicators use NPP as a proxy to determine the impact of land use on the AoP natural resources (NPP loss represents a decline in standing biomass stock). A first attempt was made by Núñez et al. (2013) to assess the impact of land use, in particular soil erosion, on the AoP ecosystem health (briefly discussed in section 7.3.2, part 2). A ratio of potential NPP (NPP_{0,i}) at grid level (10 x 10 km^2 grid cells on a world map) and the maximum average potential NPP ($NPP_{0,ref}$) an ecosystem provides worldwide (1,496 g C $m^{\mbox{-}\!2}\ yr^{\mbox{-}\!1})$ as a reference is used as a CF. These CFs are then multiplied with the SOC loss inventory flow. A world map shows the $NPP_{0,i}/NPP_{0,ref}$ ratio, i.e. CFs ranging from 0 to 1. As a result, (exergy-based) CFs for different types of land occupation were missing. Moreover, questions may arise about the usefulness of normalizing local NPP₀ values with the highest NPP an ecosystem has worldwide as this does not determine the impact land use can have on a specific location.

7.4.3 Novel NPP-based LCA indicators to assess land use impacts on the AoP ecosystem health

In trying to avoid shortcomings from previously discussed indicators, two advanced LCIA indicators are developed in this study, both based on the quantification of NPP losses due to land occupation, i.e. NPP is used as a proxy to assess impacts on ecosystem health (Table 15). Figure D.2 shows the cause-effect chain of the proposed indicators. Both indicators are developed to assess the same type of land use impacts, however, the underlying structure of the indicators is different. As shown in Figure 33, the actual loss of NPP can be calculated on the basis of two concepts: HANPP and naturalness (or hemeroby). For both indicators, spatially differentiated CFs are calculated in exergy terms. Conventional exergy analysis in LCA is particularly focusing on the AoP natural resources instead of ecosystem health (Achten et al., 2008). In this context, it is innovative to calculate land use impacts on the basis of exergy flows in ecosystems. A [0-1] approach for several land types was applied when calculating the CFs, i.e. the value of semi-natural NPP in human-induced systems is taken into account (NPP left after human intervention is still valuable for ecosystems).



Figure 33 Representation of the HANPP and hemeroby concepts to assess the loss of NPP as a result of land use compared to the potential natural NPP (NPP₀) at a specific location. Abbreviations: PNV is the potential natural vegetation; NPP_t is the remaining NPP in the ecosystems after land conversion and possible harvest.

Table 15A comparison between different LCIA indicators that assess the loss of NPP as a proxy for determining the impact of land
occupation on regulating and supporting ecosystem processes. Both the innovative aspects as the limitations of each
indicator are discussed.

	Alvarenga et al. (2013)	Alvarenga et al. (2015)	Núñez et al. (2013)	This study
I N O V A T I O N	 * First indicator that assesses land use impacts based on an <i>potential</i> NPP loss within the AoP <i>natural resources</i> * Spatially-differentiated CFs calculated in exergy terms * CFs for all land occupation flows as proposed by one or more process or input-output LCA databases (specific the ecoinvent database v2.2) 	 * First indicator that assesses land use impacts based on an actual NPP loss within the AoP natural resources * Spatially-differentiated CFs calculated for 4 types of land uses * Value assigned to the remaining NPP after land use → [0-1] approach for different land types 	 * First indicator that assesses land use impacts based on an potential NPP loss within the AoP ecosystem health * Spatially-differentiated CFs 	 * Both indicators assess land use impacts based on an actual NPP loss within the AoP ecosystem health * Spatially-differentiated CFs calculated in exergy terms, with a [0- 1] approach for different land types CFs based on the hemeroby concept (naturalness) and directly applicable for process-based LCA (ecoinvent database versions 2.2, 3.0 and 3.1) CFs based on the HANPP concept (40 land use types are available)
L I M I T A T I O N	* No value assigned to the remaining NPP after land use -> [0]-[1] approach for different land types	* Not expressed in exergy terms * Missing CFs for land occupation flows (other than cropland, pasture, infrastructure and wilderness) as proposed by one or more process or input-output LCA databases	 * Not expressed in exergy terms * Missing CFs for land occupation flows * No value assigned to the remaining NPP after land use * Ecosystem with the highest potential NPP worldwide as a reference 	 * Hemeroby concept: CFs calculated based on a qualitative (subjective), ecological framework * HANPP concept: CFs not directly applicable for LCA as the amount of land occupation flows of process or input-output databases are inadequate or the flows do no match due to different background land use classification systems

Material and methods HANPP approach

The actual loss of NPP can be calculated on the basis of HANPP, which is a more quantitative, socio-economic approach compared to the hemeroby concept. In addition to the work performed by Alvarenga et al. (2015), additional *exergy-based* spatially differentiated CFs are calculated based on the HANPP concept (as explained in section 7.3.2, part 1), but now for 40 land use types instead of 4 and 169 countries instead of 162. Therefore, a world map with 3680 zones was created in the software ArcMap 10 through intersection of the 'world countries' map available in the ArcGIS software and 'the land use systems of the world' map (LADA, 2008) that contains 40 types of land uses (e.g., shrubs, agriculture, urban, bare areas, Figure 34). A pixel of this map represents a 10 km² square (Figure D.3).



Figure 34 Mapping of land use systems at global and regional scales (LADA, 2008)

Note that the global LADA map was chosen instead of the CORINE land cover classes that are used by ecoinvent (a commonly used LCA background database) as the latter map is only representative for Europe. One zone represents the area (part of a country) that is assigned to an overall land use type (according to the LADA classification) and a zone is named after the country followed by the land use system e.g., Belgium-Urban land and Sweden-Forest, protected.

Subsequently, each grid cell value of the open access global HANPP map of the year 2000, expressed in g C m⁻² year⁻¹, is multiplied with the biomass-to-exergy conversion factor of 42.9 MJ_{ex} kg C⁻¹, which is the average value of 13 biomes' conversion factors (Alvarenga et al., 2013). Spatial analyst tools available in the ArcGIS software were used to calculate the area-weighted averages of HANPP (MJ_{ex} m⁻² year⁻¹) within each zone (after intersection of the HANPP map and the zonal map). These averages represent the loss of NPP due to land use as a proxy for determining the impact on the AoP ecosystem health. Figure 35 represent the procedure to develop these zone-dependent CFs. NPP loss due to human activities on land can be defined as the ratio between the NPP remaining in the ecosystems after harvest (NPP_t) and the NPP of the potential vegetation assumed to prevail in the absence of land use with current climate (NPP₀), as described by Eq. 4 (Haberl et al., 2007).

$$CF_{occ,i,j} = NPP_{loss,x} = NPP_{0,x} - NPP_{t,x} = NPP_{0,x} - NPP_{act,x} + NPP_{h,x} = \Delta NPP_{LC,x} + NPP_{h,x} = HANPP_{x}$$
(16)

Where $CF_{occ,ij}$ represents the CF of land occupation impact on ecosystem health for land use type i in country j (further referred to zone x), NPP_{loss,x} the NPP loss due to land use in zone x ($MJ_{ex} m^{-2} year^{-1}$), NPP_{0,x} the average potential NPP in zone x ($MJ_{ex} m^{-2} year^{-1}$), NPP_{t,x} the average remaining NPP for the natural environment after land use in zone x ($MJ_{ex} m^{-2} year^{-1}$), NPP_{act,x} the average NPP of the actual vegetation in zone x ($MJ_{ex} m^{-2} year^{-1}$), NPP_{h,x} the amount of NPP harvested in in zone x ($MJ_{ex} m^{-2} year^{-1}$), $\Delta NPP_{LC,x}$ the impact on NPP due to human-induced land conversions in zone x ($MJ_{ex} m^{-2} year^{-1}$) and $HANPP_x$ the average NPP impact due to human land use in zone x ($MJ_{ex} m^{-2} year^{-1}$).



Figure 35 Development of zone-specific CFs (area-weighted averages of HANPP), based on the global LADA land use classification map.

Naturalness approach

Determining the NPP that is left after human interventions on land based on the naturalness approach is more directly applicable in LCA. The concept of naturalness is used as a state descriptor of ecosystems, i.e. the antipode of anthropization of the system is determined. The highest naturalness corresponds with the PNV in an undisturbed situation (Machado, 2004). Naturalness is strictly linked to ecological aspects (no socioeconomic ones) and is defined by a set of descriptive conditions such as the availability of biotic elements, water dynamics, level of land fragmentation, the presence of artificial elements, the level of biodiversity and pollution (Machado, 2004). Compared to the HANPP approach, which is a single quantifiable parameter, naturalness is too complex to be expressed in the same way. Therefore, an ordinal scale (greater, smaller, more, etc.) is used where a distinction between different land classes can be made based on these descriptive conditions (Fehrenbach et al., 2015). The concept of hemeroby is clearly linked to naturalness as it measures the disturbance by humans (or intensity of land use) and, thus, the unnaturalness of current vegetation (Brentrup et al., 2002). Wrbka et al. (2004) identified a strong monotonous and significant linear relation between HANPP and hemeroby. Hence, this approach is based on the assumption that the more naturalness is preserved, the better it is for ecosystem health.

The integration of the hemeroby concept in applicable LCIA land indicators is very limited. Brentrup et al. (2002) determined the naturalness degradation potentials (NDPs) for different land use types as described in ecoinvent version 2.2, which can be used for the calculation of CFs in LCA. A linear approach was used, i.e. ten hemeroby classes were identified and the correlated NDP values were rendered on a scale from 0 to 1 with constant intervals of 0.1 (Fehrenbach et al., 2015). In this study, we have further assigned NDPs to all land occupation flows of the ecoinvent database version 3.0 and 3.1 (mainly CORINE land cover classes), based on the hemeroby/naturalness classes and descriptions as defined in Table 1 and Appendix A of Machado (2004) and Table 1 and Annex of Brentrup et al. (2002). Up till now, there is a lack of spatial hemeroby data outside Europe (Fehrenbach et al., 2015). In this study, a new land use indicator is introduced that accounts for the share in natural NPP that is deprived due to land occupation, by multiplying countryspecific NPP_0 values with the NDP of that specific land use type (see Eq. 5). The indicator measures the artificial disturbance of the potential natural NPP state of the occupied land.

$$CF_{occ,i,j} = NPP_{loss,i,j} = NPP_{0,j} \times (1 - n_i) = NPP_{0,j} \times (NDP_i)$$
(17)

Where $CF_{occ,i}$ represents the CF of land occupation impact on ecosystem health for land use type i in country j, NPP_{loss} the total NPP loss due to land use type i in country j (MJ_{ex} m⁻² year⁻¹), NPP₀ the average potential NPP (MJ_{ex} m⁻² year⁻¹) for country j (available in supporting information of Alvarenga et al., 2013), n_i represents the naturalness of land use type i (-) and NDP_i the naturalness degradation potential of land use type i (-), which is assigned to the different levels of hemeroby (Brentrup et al., 2002).

Results and discussion

HANPP approach

The site-dependent CFs for terrestrial area occupation for 3680 zones (country and land use specific), are available in Table D.3 and are expressed as the mean NPP losses (MJ_{ex} m⁻² yr⁻¹) compared to the potential natural NPP (NPP₀) in that specific zone. The area of each zone (km^2), as well as the percentage of the total area of the country covered by a specific land type (%) and the standard deviation (STDV) of the CFs are calculated. However, when the specific location of land use is known, it is recommended to use site-specific CFs as can be found directly on the HANPP map (expressed in MJ_{ex} m⁻² year⁻¹), which is downloadable in kmz format in Appendix E (Figure 36). For example, the impact of a build-up area depends on the intensity of urbanization; e.g., Paris (the capital of France) has a higher land use impact (20.8 MJ_{ex} m⁻² year⁻¹, directly from the kmz file) than the area-weighted CF for all urban areas in France (16.1 MJ_{ex} m⁻² year⁻¹, Table D.3).



Figure 36 The HANPP map (expressed in MJ_{ex} m⁻² year⁻¹)

In contrast to the hemeroby approach, it is possible to obtain negative CFs, e.g., man-made ecosystems may have a higher NPP than the potential natural ecosystems on the same location. One example is large scale irrigation in agriculture in Algeria, which leads to a negative CF of minus 2.41. Occasionally, managed ecosystems can have a higher 'productive value' than ecosystems can reach in the absence of human intervention. However, it can then be appropriate to combine NPP with other indicators, e.g., indicators that assess the impact on biodiversity or the impact on fresh water funds due to irrigation (Weidema and Lindeijer, 2001).

Because the HANPP concept as described in this study makes use of the LADA land use classification, it is difficult at the moment to couple it with commonly used background databases in process LCA such as ecoinvent, which is based on CORINE land classes. For example, 'crops and moderate intensive livestock density' (LADA land use class) is not completely compatible with 'occupation, annual crop, non-irrigated, intensive' from the CORINE classification. Apart from process-based LCA that often requires many assumptions and decisions that can make life cycle assessment a very complex and time consuming endeavor, an alternative has been presented in literature to calculate the inventory of systems at meso (e.g., sector) and macro level (e.g., country) based on a sectorial exchange of mass or money, i.e. performing an environmental input-output analysis. Even the most

detailed input-output databases (e.g., Exiobase2) contain only a limited amount of land occupation flows (e.g., only arable, forest and pasture land use) without an indication of the intensity of the land use (Exiobase, 2007). Consequently, only the impact of these types of land uses can be taken into account, i.e. for now, the amount of land flow types of those databases are insufficient to fully support this framework. It is therefore recommended that these databases expand the amount of occupation flow types in the future.

Another point of attention is the low resolution of the zonal map (1 pixel represents 10 km²) as this may add to uncertainty of the final results (CFs). During the development of the LADA land use map, which is used as the basis of our work, access to relevant data and data quality was a major concern. Several global data layers of variable quality with different resolutions / scales were put together, which is a risky exercise. An intermediate solution corresponding to a tradeoff between data accuracy and availability was retained. The rather low resolution of the LADA map and the choices made/data used to create the respective map may lead to the fact that the calculated CFs per land use type within each country not entirely reflect reality (e.g., the demarcation of the zones could be more precise, which would lead to more accurate CFs) and the occupation flows may be difficult to interpret (e.g., forest - with moderate or higher livestock density are not common in Belgium; forestry and livestock production are mostly separated). We would emphasis the fact this is a first attempt in the LCA community to develop such an amount of zonal specific land use CFs to be able to account for local impacts and progress can be made when more accurate and higher quality data regarding global land use systems become available.

Furthermore, questions may arise about the intrinsic value of NPP_t for ecosystems (e.g., biomass left over after harvest is considered to have an equivalent value as potential natural biomass). To date, there is no information on how to account for a potential difference in intrinsic value for nature. Therefore, more research (e.g., data monitoring and combining expertise from different disciplines) in this field is recommended.

Naturalness approach

The area-weighted NPP₀ values are available in the supporting information of Alvarenga et al. (2013) and the NDPs per land use type can be found in Table D.2. When multiplying the country-specific NPP₀ values with the NDP of a specific land use type (Eq. 5), the CF for land occupation is calculated. However, to calculate site-specific land use impacts, the world NPP₀ map expressed in exergy terms is available in Appendix F (kmz format). For example, NPP₀ in the region of Plymouth (city in the south of the United Kingdom) amounts to 29.5 MJ_{ex} m⁻² year⁻¹, which is significantly higher than the average area-weighted value of 23.2 MJ_{ex} m⁻² year⁻¹ for the United Kingdom, i.e. detailed information about the exact location can result in a more accurate CF.

The naturalness approach has the advantage that the exergy-based spatially differentiated CFs are linkable with the elementary flows of ecoinvent database versions 2.2, 3.0 and 3.1, i.e. directly applicable for process-based LCA. However, this implies that there is no direct compatibility with other background databases yet. Furthermore, the naturalness assigned to a certain land use type is based on a qualitative (subjective) framework, which can be interpreted as a drawback of this indicator when used in LCA studies (Reif and Walentowski, 2008).

7.5 Conclusions

Human impacts on land and more in particular on our natural ecosystem processes and available land-based resources continue to grow as the pressure of a rising population takes a huge toll on the environment. In an attempt to quantify possible impacts of land use, several LCIA indicators have been developed recently. An overview of these indicators is provided within a framework of impact pathways linked to land use. Related to land's natural resources, solar energy or NPP is regularly used as a proxy indicator for assessing a change in standing biomass stock (e.g., the CEENE method) or soil depletion. The impact on other land resources (abiotic ones), such as depletion of minerals, are often not linked to an impact category land use but are assessed separately. Concerning the AoP ecosystem health, most indicators are developed to assess the loss of biodiversity due to land use in terms of species richness loss. In this study, an advanced NPP-based proxy indicator is developed in attempt to assess the impact of land use on environmental ecosystem processes. Because a regionalized assessment is required in LCA, site-specific CFs for different types of land use are developed based on the loss of natural NPP expressed in exergy terms. The concepts of HANPP and hemeroby were used to determine this NPP loss. In the end, both LCA indicators allow for a better assessment of the environmental impact of occupying different types of land, e.g., it is now possible to highlight the lower fertile land footprint of marine microalgae production compared to most terrestrial crops.

PART IV Conclusions and perspectives

Chapter 8 Overall conclusions

8.1 The potential of algae: towards commercialisation

Algae have attracted considerable interest globally because of the apparent potential as a feedstock for a bio-based economy. It's a fast-growing type of aquatic biomass, able to convert readily available resources such as sunlight, carbon dioxide, nitrogen and phosphorous, often obtainable from waste streams or available in excess in coastal areas, into valuable biomass components to be used in a variety of industrial sectors (e.g., food, cosmetics, energy), as shown in Figure 37.



Figure 37 Example of a microalgae production scheme, indicating the main market opportunities: nutraceuticals and medicinals, food and feed, personal care products, chemicals and fertizers and energy.

Due to the concern regarding limited energy sources caused by the rapid growth in human population and industrialization, interest in renewable and sustainable energy production increased. While first generation biofuel crops and production technologies are quite efficient, their production is often seen as unsustainable as they compete with food crops for available land and fresh water. The goal of second generation biofuel processes is to increase the amount of biofuel that can be produced sustainably by using non-food feedstocks, biomass that is left behind once a food crop has been extracted and waste streams from industry such as woodchips and olive pulp. However, these biofuels are not yet produced on a commercial scale. The challenges include cost reductions, technological breakthroughs, infrastructure needs and a continuous supply of biomass (Antizar-Ladislao and Turrion-Gomez, 2008). Therefore, over the past 10 years, tremendous efforts have been made to produce (marine) algae, which can be cultivated in open seas or on marginal land, for fuel applications such as biodiesel, bio(syn)gas, bioethanol and biohydrogen (third generation biofuels). However, at the moment, the production of these low value algae-based products generally performs worse compared to the existing (petroleum based) alternatives because the energy embodied in the algal fuel is lower than the energy required to produce it. Technical challenges remain in the upstream (cultivation) and downstream (harvesting, drying and extraction techniques) processes to improve the biomass yield and energy efficiency of the entire algal supply chain.

Beyond the technical challenges and environmental constraints, the economic feasibility of algal cultivation and associated biofuel production technologies is low. A few technoeconomic studies (Amer et al., 2011; Davis et al., 2011; Delrue et al., 2012; Richardson et al., 2010; Norsker et al; 2011; Chauton et al., 2015; Draaisma et al., 2013) revealed that microalgal cultivation costs for biofuel production in Europe fluctuate from €2.2 to €12.7 per kg DW biomass, depending on the climatic conditions, the type of cultivation systems (production in open ponds is cheaper) and the production scale and whether or not wastewater treatment is the main goal. Cost estimates of fuel production from algae found in literature varied widely, ranging between €0.40 and €7.37 per liter, due to different assumptions made, often based on small scale observations (Quinn and Davis, 2015). The estimated cost for biodiesel produced from soybean and rapeseed oil in 2012 was €1.19 L⁻¹, from palm oil in Malaysia and Indonesia €0.91 L⁻¹ (International Renewable Energy Agency, 2013), and from waste cooking oil €1.20 L⁻¹ (Mohammadshirazi et al., 2014) compared to €0.24 L⁻¹ for conventional diesel, which is even lower today. At least, a production cost of €0.68 kg DW⁻¹ should be achieved to become competitive with currently used feedstocks for biodiesel production (Norsker et al., 2011). Consequently, the focus has shifted toward higher value applications, whether or not in combination with waste stream mitigation and optimal valorization of by-products. Over the last decades, the strong increase in prices for key ingredients of the feed industry such as fish meal, fish oil and soybeans (mainly as a result of increased demands and low supply) makes it interesting to produce

algae for the feed market (Rana et al, 2009). This involves higher production costs (more control needed, preferable a PBR installation) but due to the growing demand for high-quality and nutrient-rich algal biomass in the feed industry, the selling prices also increase to up to \in 300 kg⁻¹ DW, dependent on the type of algae and its quality (personal communication with experts in the field).

Nowadays, most seaweed and microalgae producers are located in Asia, the USA and Australia, primarily to serve the food and feed industry, but recently also some European companies are attempting to produce higher-value algal ingredients (e.g., AlgaSpring BV, Neoalgae, Proviron Industries NV). Algae production might improve the European competitiveness and reduces the dependence of non-EU countries for e.g., protein rich biomass that is highly used in the feed and food sectors. However, Europe's main weaknesses such as the exposed coastlines, suboptimal climatic conditions, high labour costs, strong regulation concerns (complexity of the feed (EC 68/2013, EC 767/2009, EC 91/271) and food (EC 258/97, EC 629/2008) regulations) and low internal demand (market) for algal products request a high priority in R&D funding and co-operation between academia and industry to overcome the main hurdles in the commercialization of algae-based products. The strong position in science and technology, the available technical skills and industrial and governmental interest in algal products are the main ingredients to enhance its position in algal research and to stimulate a more environmentally friendly and economically feasible production.

8.2 Microalgal biomass production for the feed industry

Due to the increase in global population, there is a great need for more food products, including animal products, and hence sustainable protein-rich feed ingredients, moving away from e.g., fish meal and soybeans. A first part of this dissertation investigates the potential of microalgae in the feed sector; in its early phase of development in Europe, it is essential to consider the environmental footprint of the algae production chain as it provides an opportunity to make adjustments in a timely manner. The first case study

(chapter 4) reveals the environmental sustainability of dried Nannochloropsis cultivated in ProviAPT bags in Belgium for aquaculture purposes. A production process (pilot 2012, 240 m^2) was assessed and compared with two upscaling scenarios (pilot 2013, 1320 m^2 and first production scale 2015, 2.5 ha). A note should be made that, now in 2016, the production capacity is approximately 3200 m² of PBRs, which is a lower cultivation scale than predicted in the hypothetical scenario of 2015. Contamination with bacteria, protozoa or another species of algae is a serious problem for monospecific/axenic cultures of microalgae and it has slowed down the scaling up. However, the delayed scale up did not affect the overall LCA conclusions of the study as the hypothetical scenarios could be expected a few years later than originally planned (2013 and 2015). However, it did not affect the overall conclusions of the study. The LCA showed an improvement in resource efficiency after upscaling: 55.5 $\rm MJ_{ex,CEENE}~MJ_{ex}^{-1}$ DW biomass was extracted from nature in 2012, which was reduced to 21.6 and 2.46 $MJ_{ex,CEENE} MJ_{ex}^{-1}$ DW in the hypothetical 2013 and 2015 scenarios, respectively. Upscaling caused the carbon footprint to decline by factor 20 (0.09 kg $CO_{2,eq}\ MJ_{ex}^{-1}$ DW in 2015). Compared to the footprint of a traditional fish feed, composed out of, amongst others, fish meal, soybean meal, fish oil and rice bran, which amounted to 7.70 $MJ_{ex,CEENE}$ and 0.05 kg $CO_{2,eq}$ per MJ_{ex} DW), it appeared that only the last hypothetical scenario would be competitive. Consequently, major research efforts are needed to develope a more energy efficient production system operatable under European's climate conditions.

Chapter 5 investigates the potential of an integrated algal biorefinery compared to a linear economy, producing both the same basket of products. A biorefinery where algae are produced close to an industrial facility, which delivers its own products, and where waste streams (heat, waste water and flue gases) are used to stimulate the algal growth, could be a promising concept as it is beneficial for the joining industries: on the one hand, the required products are made, and on the other hand, sustainability-related issues such as land occupation, fossil fuel use and greenhouse gas emissions can be mitigated. In addition, when algae cultivation and processing are positioned near its sale market, e.g., livestock production, it might create a win-win situation for all the stakeholders. However, according to the LCA results of the specific case study on *Scenedesmus* and *Chlorella* production as a livestock feed ingredient, the linear soybean economy still performs better

(factor 100) compared to the integrated algal biorefinery when the total resource footprint is considered. The main reason is the resource/energy-demanding algal cultivation stages, especially for blowing flue gases into the ponds. The share of energy consumption, i.e. fossils, nuclear and abiotic resources, during soybean cultivation is much lower, as mainly land resources are used for soy production (relative percentages). The rather high differences in the resources consumed could be expected because a comparative study is conducted between a mature, large-scale technology (soybean meal/oil production) and a young, pilot-scale (500 m²) process chain (microalgae meal/oil production) that is still under development.

Despite the potential of algae being used in the feed industry, further progress must be made as it faces severe problems regarding costs and environmental pressure because of the energy intensive processes along the production chain. Algae based technology in north west Europe must overcome these hurdles before it can compete with the currently existing alternatives.

8.3 Towards a better accounting for the occupation of marine and terrestrial surfaces

LCA can provide a tool to show the directions towards which technology should develop, to overcome bottlenecks and improve the environmental sustainability of marine production systems. To make a more fair comparison between the environmental footprint of algae and that of terrestrial plants, it is important to develop a methodology able to assess the impact of occupying coastal areas (e.g., for nearshore seaweed production) and/or the use or marginal land (e.g., for microalgae cultivation). This way, a better evaluation of the possible advantages of algae production over terrestrial plants, requiring fertile land, can be performed.

In chapter 6, the CEENE method was amended to additionally account for marine area occupation, either on realms, provinces and ecoregions level. This makes it the first LCIA

method able to quantify resource-related impacts associated with the use of the marine environment. The CFs were based on the exergy amount of potential NPP in the upper layers of the ocean, which is (partly) avoided by the occupation structures, thus allowing to consider the three-dimensionality of the oceans. Advanced world marine NPP maps were created based on the Eppley-VGPM model. An occupation factor α was proposed in case only a part of the euphotic zone was occupied. Also, temporally differentiated CFs are calculated for each quarter of the year as the NPP production differs among the seasons. Furthermore, average CFs expressed in MJ exergy per kg fresh or dry weight were developed for fish, mollusks, crustaceans and seaweed that can be extracted from natural marine systems.

A case study was carried out on the natural resource footprint of seaweed production in North West Europe and the sea surface occupation factor α for the single longline and raft system was determined. As could be expected, the raft system occupied more sea surface than single longline structures, which increased the consumption of marine resources (higher α factor). Compared to the results from the microalgae case studies, it can be concluded that, from an environmental resource point of view, seaweed has more potential than microalgae to be produced in a sustainable way in NWE. However, this conclusion is based on current pilot scale knowledge and hypothetical up-scaled plant scenarios. In addition, the LCA results of the case-study revealed that the resource footprint of seaweed cultivation in Ireland is already comparable with the footprint of several terrestrial plants (sugar beets, maize and potatoes). However, a careful interpretation is required as the composition (incl. dry weight content) and functionality of the different biomass types are not identical. Nevertheless, this could not be concluded for the French case. As the raft systems used near the coast of France are subject to friction, a large amount of seaweed biomass is lost which results in a much lower biomass yield (factor 5) and there is a higher demand for infrastructure compared to the single long lines used near the coast or Ireland. This, of course, has a significant impact on the overall LCA results. With respect to the type of resources used, more fossil resources are consumed during marine biomass production while more land resources are used for terrestrial biomass production. It seems that marine biomass meets the requirements to reduce pressure on land.

Chapter 7 examines how to account for different types of land occupation (e.g., urban, agricultural, forests, non-arable) in LCA. Terrestrial land and its resources are finite, though, for economic and socio-cultural needs of humans, these natural resources are further exploited. It highlights the need to quantify the impact humans possibly have on the environment due to land use. The possibility of cultivating microalgae on bare, marginal lands could be a tremendous advantage over terrestrial plants and should be assessed accordingly. As a starting point, the anthropogenic land use activities, which may be mainly socio-culturally or economically oriented, are identified in addition to the natural land-based processes and stocks and funds that can be altered due to land occupation. Already several LCIA indicators have been developed in an attempt to evaluate certain environmental impacts of land use. These indicators where briefly reviewed and categorized according to whether they assess a change in the asset of natural resources for production and consumption or a disturbance of certain ecosystem processes, i.e. ecosystem health. Related to land's natural resources, solar energy or NPP is regularly used as a proxy indicator for assessing a change in standing biomass stock (e.g., the CEENE method) or soil depletion. The impact on other land resources (abiotic ones), such as the depletion of minerals, is often assessed separately. Concerning the AoP ecosystem health, most indicators are developed to assess the loss of biodiversity due to land use in terms of species richness loss. Based on these findings, two enhanced proxy indicators are proposed. Both indicators use NPP loss (potential NPP in the absence of humans minus remaining NPP after land use) as a relevant proxy to primarily assess the impact of land use on ecosystem health. As there are two approaches to account for the natural and productive value of the NPP remaining after land use, namely the HANPP and hemeroby (or naturalness) concepts, two indicators are introduced and the advantages and limitations compared to state of the art NPP-based land use indicators are discussed. Because a regionalized assessment is required in LCA, exergy-based spatially differentiated characterization factors (CFs) are calculated for several types of land use (e.g., pasture land, urban land).

This dissertation brought insight in the potential of algae to be produced (as a feed ingredient) in an environmentally sustainable way and provided advanced LCIA indicators in an attempt to better quantify the environmental impacts of algae production by taken

into account the occupation of sea surface and different types of land. However, a further development of, on the on hand sustainable algae production processes, and on the other hand a better accounting of the environmental impact within the LCA framework is highly recommended.

Chapter 9 Perspectives

9.1 Algae production challenges

Because algae production in Europe is a young technology, many steps within the production chain can still be optimized, e.g., strain isolation, nutrient sourcing and utilization, production management, harvesting, coproduct development, pigment extraction, refining and residual biomass utilization. The major challenges include increasing the biomass concentration and reducing the energy requirements, especially at the cultivation and drying stages. Improved engineering solutions will have a significant impact on algae production. These improvements can include engineering of metabolic pathways (genetic modification) and efficient strategies for nutrient circulation and light exposure in combination with the development of low-cost scalable cultivation systems. Identifying the key environmental factors influencing the yield and biochemical composition of algae is an additional challenge. Parallel to this research, the harvesting, dewatering and processing steps need further optimization. Many technologies have already been successfully demonstrated but are relatively expensive, either in terms of equipment needed or energy required.

Breakthroughs that may allow algae to play an essential role in meeting future demands for e.g., food and energy will include the selection of highly productive (marine) species and improved cultivation and processing methods and the use of renewable energy to drive them. Also, producing algae in a biorefinery, delivering low-volume/high-value (e.g. pigments) and high-volume/low-value products (e.g., fuels), possibly in combination with waste stream mitigation, could be a way forward. Because the algal technology is rather new, many techno-economical and environmentally sustainable improvements are still possible but more research and cooperation is necessary to provide full scale algae production in Europe. Security of funding for R&D, knowledge transfer between academia and industry, identifying potential cultivation sites and clarity about all regulations and marketing of algal products are key enablers to realize a strong and sustainable algal industry. Proof of concept will have to be provided to see if larger-scale production, in combination with technology development, can lead to sufficiently low costs to be able to address e.g., the feed market.
Figure 38 provides an indicative timescale to commercialization of algal products or functions in the United Kingdom (which may give an indication for Europe). In the short to medium term, bioremediation linked to feed and fertilizer production, high value products (e.g., bioactive ingredients) of both micro- and macroalgae and consulting and technology provisioning industries are the most promising sectors. In the longer term, integrated algal biorefineries and the production of novel biomass ingredients through bioprospecting and metabolic engineering (to be used in the pharma, cosmetic or food industry) may find its way to commercialization.



Figure 38 Indicative timeline (short term, medium term and long term) to commercialization of algal based products (bioactives, food and feed, chemicals, bioenergy) and utilities (bioremediation, knowledge industries) (Schlarb-Ridley, and Parker, 2013).

On top of the knowledge gained on the main challenges involving sustainable algae production in NWE, some specific examples of research gaps could be identified from the case studies. To increase microalgal productivity, which is required to reduce the overall footprint, it is possible to increase the temperature as this would have a positive effect on the algal growth (Aleya et al., 2011; Chalifour and Juneau, 2011). However, when more hot flue gases (C-source) and/or hot water is pumped to the cultivation units, more energy use, evaporation losses and pH fluctuations will occur (which can hamper algal growth). Therefore, more experiments (trial-and-error) are necessary to achieve optimum biomass yield at a minimal environmental impact.

Both case studies on microalgae production for feed applications revealed that it is possible to meet a resource footprint within the same order as the footprint of the currently existing alternatives (traditional fish feed and soybean meal additive as a livestock feed ingredient). Therefore, electricity should be produced making use of more renewables (wind energy), more energy-efficient blowers and pumps must be installed that have fewer operating hours, and biomass productivity should increase (genetic modification could be an option) together with the amount of biomass that can be harvested. Even more possibilities to lower the resource footprint of algae production occur: the centrates can be recycled and pumped into the cultivation systems, marginal land should be used at all times to produce the biomass (suitable sites need to be identified) and further integration of the algae production in the aquaculture or livestock industry by using waste streams (manure and urine from cattle or wastewater from fish farms) as nutrients to feed the algae would be a step forward. In the case study as explained in chapter 5, the liquid fraction of the digestate that is produced within the biorefinery contains all necessary nutrients for stimulating microalgae growth. However, inducible from a small-scale experiment at the site, additional research (e.g., the work of Marcilhac et al., 2014, 2015) is necessary to determine the effect of these nutrient sources on the growth rate, as the variability and turbidity of these flows may distorts the light penetration and the possible presence of heavy metals could negatively affect the algal growth. Additionally, the electricity and surplus heat that is produced within the biorefinery could be used for algae cultivation and processing, and it would lower the resource footprint of the system. However, this approach hinders a direct comparison with the footprint of the soybean-based linear economy because the (amount of) functionalities obtained from the basket of products would no longer be the same.

Furthermore, it appears possible at first sight to leave out the energy-intensive drying process and directly feed the animals with wet algal paste (possibly after centrifugation). However, the risk of instability, contamination and non-availability of the biomass will increase.

It has been proven that some algae species have beneficial effects on animal health. To substitute e.g., soybean meal which is an key protein source in the feed sector, several algae species (e.g., Chlorella, Nannochloropsis, Spirulina, Isochrysis) are good candidates as they have an optimal nutritional profile (Enzing et al., 2014). However, due to the scarce availability of experiments and conflicting results, more research is necessary to determine whether the amino acid profile and protein efficiency ratio (i.e., digestibility) is similar to or better than soybeans. Moreover, one should wonder what the capability of livestock/fish to tolerate (a full) replacement of soybean/fish meal with algal meal is. Factors causing intolerance might include the high ash content of the algae or amino acid imbalances (Lum et al., 2013). Recent studies of Tulli et al. (2012) and Tibaldi et al. (2015) indicate that a replacement of up to 20% crude protein from fish meal and 36% fish lipids do not hamper feed intake or growth performance of fish. However, additional research is necessary to determine the digestibility and nutritional value (exergy content is already a first indication) of algae that are produced to be a feed ingredient. Furthermore, it seems possible to feed livestock with dried algal biomass without the extraction of oil. However, recent studies have indicated that the total amount of fat should be limited to \pm 6% in animal feed (DM). Otherwise, too much fat (especially unsaturated fatty acids) can cause problems for the digestive system.

In chapter 6, an LCA study of seaweed production in NWE is performed. When comparing the resource footprint of nearshore seaweed cultivation in Ireland with the footprint of microalgae and several terrestrial plants (e.g., maize) produced in Europe, it could be concluded that seaweed production is already quite efficient and competitive. As it is expected that the energy mix will become more renewable, it is anticipated that the footprint of seaweed production will be even lower in the future. At that point, seaweed could be cultivated as a sustainable biomass in (North West) Europe as it avoids much of the competition for land and fresh water. However, the comparison between aquatic and terrestrial biomass assumes an equal functionality of both types of biomass. Though, seaweed biomass has at the time of harvest a higher moisture content (± 10% DM) than the terrestrial crops (approx. 24%). This implies effects in the processing, when e.g., more drying of the seaweed biomass is required. When valuable data of efficient processing steps become available, more research will be required to fully quantify the life cycle footprint of the whole process chain. Also, further research focusing on enhanced environmentally friendly nearshore and offshore seaweed cultivation techniques (enabling high biomass yields) is necessary. For example, the raft systems used near the coast of France were subject to friction, which resulted in a large amount of seaweed biomass being lost. However, previous experience suggests that the use of single longlines is not an option due to the turbulent marine conditions close to the harbour of Lézardrieux. Additional work in marine engineering may contribute to the development of suitable cuitivation techniques. Furthermore, additional effects of seaweed production on the environment (emissions, biodiversity, nutrient bioremediation etc.) and the economic feasibility should be assessed and compared to the existing alternatives. For example, the integration of seaweeds into marine fish aquaculture systems could be a key step toward sustainability, long-term profitability, and responsible management of coastal waters.

9.2 LCA challenges

LCA is an increasingly important tool for environmental policy and industry. When performing an LCA, methodological choices such as allocation and defining the system boundaries, time scope and functional unit have to be made. This may hinder a fair comparison of the results and makes it more difficult to draw overall conclusions. In addition, data availability, data quality and types of data used in the assessment are the focus of discussion for many LCA studies. Due to the lack of data from large scale operative plants, the studies of algae plants are often based on conceptual designs and assumptions and in the best case on pilot scale data, which adds to uncertainty. LCA is a viable screening tool that can pinpoint environmental hotspots in complex value chains. However, the fact that we have to make a choice out of the range of existing LCA methods implies the incompleteness of grasping all relevant environmental impacts. Future advances in LCA in enhancing regional detail and accuracy as well as broadening the assessment to economic and social aspects will make it more relevant for the end-users.

The fact that algae can be cultivated on marginal, non-arable land or at sea is an enormous advantage because (fertile) land is today a scarce resource. However, accounting for the environmental benefit of using marginal land instead of fertile land used by other types of biomass (e.g., food crops) or the use of sea surface is not aphoristic. The assessment of the potential environmental impact associated with terrestrial land use (or land occupation) has gained already wide attention in LCA. Nevertheless, efforts still have to be made to account for the environmental impact of different land uses in a consistent way. Should we focus on accounting for resource depletion related to land use or on the impact it may have on ecosystem functioning (or both) and should this be assessed by one LCIA method or a combination of several? Also, a more detailed guideline and better understanding of global land use impact assessment in LCA is required. In chapter 7, two enhanced proxy indicators are developed to assess the environmental impact of different types of land use on the AoP ecosystem health. The indicators are based on different concepts: HANPP and hemeroby (or naturalness). However, as both concepts are rather new in LCA, further research may contribute to a better understanding and implementation of these concepts in sustainability assessment. Especially more detailed global land use classification systems (e.g., LADA) may increase the preciseness of results. Another challenge is to simultaneously evaluate multiple economic, environmental and social impacts of land use that are expressed by a variety of different indicators as no indicator describes a full range of impacts caused by land use. Therefore, a consensus should be reached on which kind of land-use impacts must be assessed and on how to combine the different indicators. In addition, a more in-depth analysis of the links between different ecosystem processes and the relation with biodiversity is strongly recommended. These complex natural interactions may be difficult to grasp within the current LCA framework, i.e. a commitment to continuously improve the framework is highly important (Pérez-Soba et al., 2008). In most cases, the lack of reliable data limits validation and cross-comparison and hinders the application of certain land use indicators in LCA. There is also more research required related to the LCA land-use assessment framework, because the potential natural NPP can be questioned as a reference baseline due to the fact that there

may be multiple equilibria for the final state (Othoniel et al., 2015). Furthermore, for transformation and permanent impacts, it remains unclear how long it takes to recover from land use change, which can be a very dynamic process. Up till now, there is no widely accepted LCIA method that covers all land use impacts. Such an effort is multidisciplinary and requires engagement of many experts in related fields.

As the assessment of terrestrial land use (or land occupation) in LCA has attracted considerable interest over the past years, it can be expected that the same efforts will be made to quantify the impact of extracting marine resources or occupying sea surface. However, accounting for the impact of using sea surface (e.g., seaweed farming) in LCA is even a more difficult task. The study of Taelman et al. (2014) provides a LCA method (CEENE 2014) as a first attempt to quantify the impact of marine areal occupation. Offshore or nearshore seaweed production systems have to a certain degree an environmental impact related to the use of sea surface due to the fact that the infrastructure used at sea may interfere with the availability of sunlight for NPP production. Therefore, the complexity of the marine environment should be fully taken into account. Additional research and experimental data are required on light scattering, turbulent conditions, the way seaweeds are positioned in the water column and the light permeability of seaweed blades. Furthermore, the effect of increasing amounts of greenhouse gases in the atmosphere (anthropic climate change) on NPP production in marine areas is expected to have diverse impacts. A direct effect is rising ocean surface temperatures, which leads to enhanced stratification of the water column; it suppresses nutrient exchange through vertical mixing and upwelling near the coast. Due to the observed coupling of physical and biological functions in marine systems, it is very complex to predict all possible effects. Most models expect a decline of marine NPP (and marine biodiversity) as a possible future change caused by global warming (Behrenfeld et al. 2006; Steinacher et al. 2010). Though, more research seems essential to fully comprehend the climate-driven trends in ocean productivity. In a further study, the influence of predicted changes in e.g., sea surface temperature could be assessed or a sensitivity analysis might be performed to highlight its influence on marine NPP and the impact of marine area occupation.

Moreover, life cycle inventory databases such as ELCD and ecoinvent do not support in full the framework proposed in this study because reference flows from oceans are lacking and/or there are no modeled processes using such reference flows. Therefore, four reference flows (fish, seaweeds, crustaceans and mollusks) for natural marine systems are proposed for implementation in life cycle inventory databases. For human-made systems, it is advisable to chance the reference flows related to occupying the seabed into occupying ocean surfaces (or just add these flows to the database) because the seabed could be located below the NPP zone. After implementing these proposals, it would be possible to calculate the resource footprint caused by marine areal occupation, taking into account the three-dimensionality of the ocean by calculating the amount of NPP available in the photic zone.

Similar work has been done by Langlois et al. (2014a, 2015) to assess the impact of human activities on the marine environment. The first work is related to fishing activities, where the amount of NPP required to produce a given mass of fish in a specific ecosystem is accounted for as being no longer available for the natural aquatic environment after fishing. The second study focusses on the development of a new sea use impact category, in an attempt to assess the impacts of biomass removal, shading, seafloor destruction and artificial habitat creation. Especially the work on shading impacts is closely related to the work performed in chapter 6, i.e., assessing the avoided NPP production due to shading (sea surface occupation). However, in this PhD dissertation, extra attention is paid to the possibility of partial shading (e.g., aquaculture structures) by introducing a sea surface occupation factor α . In addition, exergy based temporal (quarter-dependent) and spatial (different scales: marine realms, provinces and ecoregions) CFs were calculated. Overall, these studies underline the importance of assessing impacts of human activities on the marine environment. However, due to the lack of relevant data it is recommended to combine fields of expertise around the complex marine environment in order to estimate the impact of human activities at sea more accurately. Therefore, a multidisciplinary approach is required and joint initiatives of research institutions, policy and industry are essential.

Apart from the challenges related to a better quantification of the impact of land or sea surface use on the environment when cultivating algae, there is a need for more LCA casestudies on algae production for applications other than biofuels. Especially for Europe and on the shorter term, there is a strong indication that algae will be cultivated for higher value applications (e.g., as a cosmetic ingredient). Moreover, data is missing on direct emissions from algae cultivation systems. Especially in the case of wastewater treatment, direct emissions from open ponds can have a significant contribution to the environmental footprint of the whole system. Therefore, more emission-related measurements are recommended to allow for a better quantification of such aspects in LCA.

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Appendix

Appendix A-F associated with chapters 5 to 7 are provided on the enclosed CD-rom.

Curriculum Vitae

1. General information

Personal details

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Professional position

2012-present Ghent University, Bioscience engineering faculty, research group Environmental Organic Chemistry and Technology (EnVOC): **PhD** (http://www.ugent.be/bw/doct/en/research/envoc)

- 2010-2012 Ghent University, Faculty of Bioscience engineering: Master in Bioscience Engineering, option Environmental Technology + minor environmental coordinator. Master thesis: 'The sustainability of algae as a resource for a biorefinery.' Promotors: Prof. Jo Dewulf (UGent, EnVOC) and Luc Roef (Product Manager bij Proviron Holding NV); tutor: dr. ir. Steven De Meester (UGent, EnVOC).
- 2007-2010 Ghent University, Faculty of Bioscience engineering: Bachelor in Bioscience
 Engineering, option Environmental Technology. Bachelor thesis:
 'Modeling of biological nitrogen removal from manure.' Promotors: Prof.
 Eveline Volcke (UGent, Biosystems control) and Prof. Erik Meers (UGent, Ecochem).

2001-2007 Sint-Bernarduscollege, Oudenaarde (Belgium): Science-mathematics (6 hr)

2. Research activities

Expertise

- Environmental sustainability, more specifically life cycle assessment (LCA) and ecosystem service assessment, their application and methodology
- Resource usage and efficiency of production systems using exergy analysis and Exergetic LCA (ELCA)
- ELCA on algae (micro- and seaweed) production systems in North West Europe for several applications (animal feed, wastewater treatment)
- Further development of Life Cycle Impact Assessment (LCIA) methods, to better quantify the impact of occupying marine and terrestrial surfaces
- Ecological modelling

Skills (other than expertise)

Teaching

Practices, exercises etc. at UGent, Faculty of Bioscience Engineering

2012-2013	<i>Clean Technology</i> (Lecturer Prof. Dewulf), exercises, tasks (12.5 hrs)
2013-2014	Clean Technology (Lecturer Prof. Dewulf), exercises, software teaching,
	tasks (18 hrs)
	Chemical analytical methods - partim Organic (Lecturer Prof. Demeestere),
	exercises (12 hrs)
2014-2015	Clean Technology (Lecturer Dr. ir. Schaubroeck), exercises, software
	teaching, tasks (21 hrs)
	Chemical analytical methods - partim Organic (Lecturer Prof. Demeestere),
	exercises (12 hrs)

Lecturing Outside UGent

Three-day Course (16-18/06/15; Escola Tècnica Superior d'Enginyeria,Universitat de **València**) on "**Environmental Sustainability Assessment**: Exergy Analysis and Life cycle assessment" for "TrainonSEC Summer school; European Industrial Doctorate on Solvent Emission Control". Supported by dr.ir. Thomas Schaubroeck.

Training

Participated in: OpenLCA software training at GreenDelta GmbH, Berlin, Germany. 20 – 21/03/2013

Software

	Level
Microsoft Office	Excellent
OpenLCA	Excellent
Simapro	Excellent
ArcGIS/ArcMAP	Excellent
Visio	Very good
Matlab	Very good
Maple	Good
Java (programmeertaal)	Good

Languages

	Read	Speak	Write
Dutch	****	****	****
English	****	****	****
French	**	**	**
German	*	*	*

Collaboration

Projects

1. Energetic Algae project (2011–2015)

The interreg Energetic Algae (EnAlgae) project is a North west European project with 19 partners and 14 observers, spread over 7 countries. The project aims at developing sustainable technologies for algae biomass production and reducing greenhouse gases (GHG). The initial objective is to grow algae biomass for energy applications. In this project, 9 algae (both microalgae and seaweed) pilot facilities are developed. Sue Ellen is involved in work package (WP) 2, action 11. This includes the responsibility for environmental life cycle analysis (LCA) of some of the pilots. The resource footprint of cultivating and processes algal biomass is determined and bottlenecks in the environmental field are identified with the intention to make the process more sustainable.

2. Omega-extract project (2013-2015)

FISCH omega-extract project of the Flemish Government: 'Downstream processing of photoautotrophic microalgae to high quality omega-3 rich algae oils on pilot scale: properties and applications'. The produced algal oil will be tested in cosmetic applications. Sue Ellen works in WP 6, where the sustainability and economic feasibility of producing microalgae for cosmetics is assessed. Both the raw materials consumption, emissions and the economic costs of the final product are identified on the basis of the results of the life cycle analysis (LCA) and life-cycle cost analysis (LCC). The most sustainable and economically viable cultivation and downstream processing will emerge from the results of these studies. Extra: participation in writing the project proposal as a first step towards project acceptance. 3. Bijzonder onderzoeksfonds (BOF) (2015-2016)

Application for a finalizing PhD grant (BOF) accepted on 05/05/2015 for a period of 8 months, starting from December 2015.

Main partners

Research Institutes
Wageningen University and Research Centre (the Netherlands)
Norwegian University of Science and Technology (Norway)
Universidade Federal de Santa Catarina (Brazil)
European Commission – Joint Research Centre (Italy)
Centre d'Etude et de Valorisation des Algues (France)
National University of Ireland, Galway (Ireland)
KU Leuven Kulak (Belgium)
Instituut voor Landbouw en VisserijOnderzoek (ILVO) (Belgium)
Karlsruher Institut für Technologie (KIT) (Germany)
Companies
Proviron Holding nv (Belgium)
Eco Treasures NV (Belgium)
GOVA benelux (Belgium)
VITO NV (Belgium)

Publications and presentations

Peer-reviewed articles in international journals (A1)

- 1. **Taelman, S.E.**, De Meester, S., Roef, L., Michiels, M., Dewulf, J. (2013). The environmental sustainability of microalgae as feed for aquaculture: A life cycle perspective. *Bioresource Technology* 150, 513–522.
- Taelman, S.E., De Meester, S., Schaubroeck, T., Sakshaug, E., Alvarenga, R., Dewulf, J. (2014). Accounting for the occupation of the marine environment as a natural resource in life cycle assessment: an exergy based approach. *Resources, Conservation and Recycling* 91, 1–10.
- 3. **Taelman, S.E.**, De Meester, S., Van Dijk, W., Da Silva, V., Dewulf, J., (2015) Microalgae as a protein rich livestock feed ingredient in the Netherlands: an environmental sustainability analysis. *Resources, Conservation and Recycling* 101, 61–72.
- 4. Sfez, S., Van Den Hende, S., **Taelman, S.E.**, De Meester, S., Dewulf, J. (2015) Environmental sustainability assessment of a microalgae raceway pond treating

aquaculture wastewater: From up-scaling to system integration. *Bioresource Technology* 190, 321–331.

- 5. **Taelman, S.E.**, Champenois, J., Edwards, M. D., DeMeester, S., Dewulf, J. (2015) Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe with a focus on quantifying sea surface occupation. *Algal Research* 11, 173–183.
- 6. **Taelman, S.E.,** Schaubroeck, T., De Meester, S., Boone, L., Dewulf, J., (2016) Accounting for land use in life cycle assessment: the value of NPP as a proxy indicator to assess land use impacts on ecosystems. *Science of the Total Environment* 550, 143-156.

Book Chapter (B2)

 Taelman, S.E., De Meester, S., Dewulf, J. (2016) Chapter 19: Algae as promising biofeedstock; searching for sustainable production processes and market applications. In: Sustainability Assessment of Renewables-Based Products: Methods and Case Studies. Dewulf, J., Alvarenga, R., De Meester, S. (eds.). In publication, Wiley. ISBN: 978-1-118-93394-7.

Presentations on conferences and workshops

Oral presentations

- Taelman, S.E., De Meester, S., Roef, L., Michiels, M., Dewulf, J., (2013) The Environmental Sustainability of Microalgae as Feed for Aquaculture: a Life Cycle Perspective. RRB-9 Conference, Antwerp, Belgium, 5-7/06/2013.
- Taelman, S.E., De Meester, S., Roef, L., Michiels, M., Dewulf, J., (2013) The (Exergetic) Life Cycle Assessment ((E)LCA) of microalgae production as feed for aquaculture: an environmental sustainability analysis. 19th SETAC LCA Case Study Symposium, Rome, Italy, 11-13/11/2013.
- 3. Rösch, C. and **Taelman, S.E.** (2014) Environmental Life Cycle Assessment of an algae production plant in the EnAlgae project and progress towards a framework for an integrative sustainability assessment. 2nd European Workshop on Life Cycle Analysis of Algal based Biofuels & Biomaterials, Brussels, <u>Belgium</u>, 24/04/2014
- 4. Taelman, S.E., De Meester, S., Van Dijk, W., da Silva, V., Dewulf, J. (2014) Microalgae as a protein rich livestock feed ingredient in the Netherlands: an environmental sustainability analysis. 2nd International Conference on Algal Biorefinery, DTU, Lyngby, <u>Denmark</u>, 27-29/08/2014.
- 5. **Taelman, S.E.**, Sfez, S., De Meester, S., Dewulf, J., Environmental Life Cycle Assessment applied to microalgae-based technologies: Methodology presentation and case studies analysis. EnAlgae Symposium, Kortrijk, <u>Belgium</u>, 18/09/2014.
- 6. Taelman, S.E., Champenois, J., Edwards, M., De Meester, S., Dewulf, J. (2015) Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe. 21st SETAC Europe LCA Case Study Symposium jointly organized with ELCAS-4, Nisyros, <u>Greece</u>, 12-14/07/2015.

Poster presentations

 Taelman, S.E., Champenois, J., Edwards, M., De Meester, S., Dewulf, J. Comparative environmental life cycle assessment of two seaweed cultivation systems in North West Europe. Algae Around the World Symposium, Cambridge, United Kingdom, 19/03/2015.

3. Spare time and hobbies

www.algenweb.be Love to read books (thrillers) Cooking for friends and family Passion for dogs and animals in general Enjoy going abroad Sport: nothing competitive ©