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Biofuels from algae: technology options, energy balance and GHG emissions

Insights from a literature review

Stefania Rocca

Alessandro Agostini

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Abstract
During the last decades, algae received increasing interest as potential source of advanced biofuels production resulting in a considerable attention from research, industry and policy makers. We report on the current-status of technology options for the potential exploitation of algae (of both macro- and microalgae species) in the biofuels and bioenergy sectors. We presents a comprehensive review of recent advances on promising algal biofuel production pathways, in terms of technological development, opportunities and limitations to their overall effectiveness. Furthermore, we analyse the main assumptions, modelling approaches and results of the algal biofuel pathways, in terms of energy and greenhouse gas (GHG) emissions balances, considered in the LCA literature. A comparison of the performance associated to the proposed algal biofuels pathways with that found for conventional fossil fuels is also reported.

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Executive summary

The recent European Union (EU) energy strategy has called for a substantial transformation of Europe's energy system based on a more secure, sustainable and low-carbon economy, with the commitment to achieve, by 2030, at least 27% share of renewables and 40% greenhouse gas (GHG) emissions reduction relative to emissions in 1990. In this context, the EU has set a cap of 7% on the final consumption of biofuels produced from agricultural crops in favour of advanced biofuels produced from non-food materials, including algae.

In the last decades, algae received increasing interest as potential source of advanced biofuels production resulting in a considerable attention from research, industry and policy makers. Algae are expected to offer several advantages compared to land-based agricultural crops, including: better photosynthetic efficiency; higher oil yield; growth on non-fertile land; tolerance to a variety of water sources and CO_2 re-using potential. Algae cultivation can also be integrated in wastewater treatment (WWT) plants to combine the contaminant removal with biofuels production. In addition, a wide range of marketable co-products can be extracted from algae, e.g. chemicals and nutrients, along with the production of biofuels, within a biorefinery concept.

Considering the potential benefits, several European-funded pilot projects, under science-business partnerships, have been dedicated to the assessment of potential algae exploitation in the biofuels and bioenergy sectors. Despite the extensive research and investments in the last decade, no large-scale, commercial algae-to-biofuels facilities had been implemented by the end of 2015. In fact, from existing algal cultivation sites, the produced biomass is currently exploited for production of food and feed, combined with the extraction of high added-value products, such as nutritional supplements.

We report on the current status of technology options for the potential exploitation of algae (both macro- and microalgal species) as feedstocks for production of biofuels, including biodiesel, biogas and bioethanol, among others. To this end, we present a comprehensive review of recent advances on promising algal biofuels production pathways, in terms of technological development, opportunities and limitations to their overall effectiveness. We consider the main process stages of the biofuels value chains, namely:

i) biomass cultivation; ii) harvesting-dewatering; iii) lipids extraction and/or direct conversion to biofuels via chemical or thermochemical processes; v) co-products management.

Furthermore, we analyse the main assumptions, modelling approaches and results of the algal biofuels pathways considered in the life cycle assessment (LCA) literature. We highlight the energy and GHG emissions balances resulting from LCA studies, focusing on the key parameters affecting the results. A comparison of the performance associated to the proposed algal biofuels pathways with that found for conventional fossil fuels is also reported.

Depending on the species and growth conditions, algae can be characterized by a certain amount of yield and chemical composition. These are key parameters for the identification and development of energy efficient and cost-effective biofuels pathways. Given the large variety of algal strains (40,000-100,000 species) and compositions, the selection of species and growth conditions needs to be carefully designed in view of the potential biofuels and/or bioproducts options. Effective algal cultivation for biofuels production requires a combination of technical breakthroughs including cultivation parameters under different locations-specific conditions.

Considering the microscopic size and properties of microalgal strains, the development of harvesting and dewatering technologies represents a critical issue with respect to the energetic requirements and, accordingly, costs. At present, there is no comprehensive analysis on the deployment potential of optimized harvesting methods at large scale, in

terms of technical viability, environmental impacts and cost effectiveness. The analysis of most of these aspects poses major challenges to future work.

Several technology options have been proposed for algal processing to biofuels. However, these technologies have been tested only at the laboratory- or pilot-scale. The main barriers to large-scale deployment of both macro- and microalgae, include:

- High demands of key resources for algal growth, such as energy, nutrients, water and CO_2;
- Difficulty of maintaining selected species with high productivity/lipids content, in outdoor culture;
- High capital and operational costs of production;
- High energy consumption associated with both the biomass production and its conversion to biofuels;
- The availability of land with suitable characteristics, i.e. climatic conditions and resource supply;
- Technical challenges of scaling up lab/pilot scale projects and cost effectiveness.

Existing LCA studies considered hypothetical scenarios based on a mix of assumed, modelled and/or experimental data. Various LCA approaches were considered with regards to the: i) functional unit (FU); ii) system boundaries; iii) impacts assessment modelling; iv) data quality and aggregation level. Hence, it is not always possible to properly compare information available in literature, as the results from modelled systems cannot be harmonized and normalised.

A major conclusion from LCA analyses of algal biofuels pathways is that the biomass yield and chemical composition of algal strains present large variability of values, depending on the inputs and technology options for algal growth. The effects of the variation of key parameters on the LCA results should be properly addressed in a broad sensitivity analysis to provide a spectrum of model outputs deriving from possible configurations of the same pathway. The results may offer a valuable contribution to ultimately identify research priorities, optimal system configurations and potential environmental risks.

Large variations of the energy and GHG emissions balances depend on the specific technologies adopted, the system boundaries, modelling parameters and how multifunctionality is resolved, such as allocation or substitution methods for co-products management. For most pathways, without considering co-products credits, the energy consumed to produce biofuels from algae is higher than the energy contained in the biofuels itself. The most favourable results are obtained when large credits were assigned to residual lipids-extracted algal biomass, such as for the displacement of corn for ethanol production or fish feed. However, these credits are "numerically" essential to obtain positive energy balance for many pathways but they should be looked at critically. The material substituted and the amounts of credits are merely modelling assumptions which may not accurately represent what may happen in reality. Any potential co-products employment option needs to be carefully assessed, also in terms of market-mediated impacts and related uncertainty.

The demand of key resources for algal growth, such as energy, nutrients, water and CO_2, as well as the costs of algal biofuels production need to be reduced to achieve viable biofuels pathways. Techno-economic challenges and environmental impacts of algae-to-fuels strategies need to be properly assessed (comprehensive impacts assessment lack at the present) before implementing systems integration strategies leading to the deployment of the algal biofuels industry.

1 Introduction

1.1 Background

General concerns about the adverse impacts caused by the extensive consumption of fossil fuels, including resources depletion, environmental pollution and climate change, led to increasing global biofuels production [1-3].

Under the Renewable Energy Directive (RED, 2009/28/EC) [4], the European Commission (EC) promotes the use of biofuels and bioenergy to accomplish various climate and energy targets to be met in the European Union (EU) by 2020 (also known as the 20-20-20 targets). These targets include:

- A reduction in the GHG emissions of at least 20% compared to the 1990 levels;
- A final energy consumption of 20% derived from renewable sources, including biofuels and bioenergy, among others;
- A reduction in the primary energy use of 20% compared with the projected levels to be achieved by improving energy efficiency.

To guarantee the sustainable use of biofuels and bioenergy, the RED establishes mandatory sustainability criteria [5]. Among them, a minimal threshold of GHG saving from the use of biofuels of 35% has to be achieved. From 2017, the GHG emission saving from the use of biofuels must be at least 50% and, from 2018, it must be at least 60% from the use of biofuels produced in new installations [4].

In 2014, a new EU energy strategy called for a substantial transformation of Europe's energy system based on a more secure, sustainable and low-carbon economy, with the commitment to achieve, by 2030, at least 27% share of renewables and 40% GHG emissions reduction relative to emissions in 1990 [6]. In this context, the EU has set a cap of 7% on the final consumption of biofuels produced from agricultural crops in favour of advanced biofuels produced from non-food materials, including algae [7].

So far, several types of biofuels have been proposed to displace petroleum products. Biodiesel and bioethanol deriving from starch-rich food crops such as corn, sugarcane, rapeseed, palm and soybean (the so-called first-generation biofuels) are produced on an industrial scale worldwide [8, 9]. The share of first-generation biofuels accounts for almost 6% of transport fuels in EU, such biofuels are blended with conventional gasoline or diesel and used in conventional internal combustion engines [3]. In recent years, the production of biofuels from edible crops has encountered large criticism on the basis of environmental concerns related to the use of fertile land otherwise used for food production. Moreover, some crop-based fuels production systems require large amounts of freshwater and fertilizers, while exhibiting only marginal energy returns and little reduction, if any, in GHG emissions [8, 9]. Biofuels produced from crops can also contribute to direct and indirect land use changes, which cause additional GHG emissions, environmental impacts and consequences affecting food availability and price, particularly in developing countries [1, 3].

In this context, algal biomass received increasing interest as potential source for the production of biofuels resulting in considerable attention from research, industry and policy makers. Algae are expected to offer several advantages compared to land-based biomass crops, including: better photosynthetic efficiency; higher oil yield; growth on non-fertile land; tolerance to a variety of water sources (i.e. fresh, brackish, saline) and CO2 re-using potential [10-13]. Algae cultivation can be also integrated in wastewater treatment (WWT) systems to combine contaminant removal with biofuels production [14-17]. In addition, a wide range of marketable co-products can be extracted from algae (e.g. chemicals, pharmaceuticals and nutritional products) along with the production of biofuels, within a biorefinery concept [18-22].

Considering the above mentioned benefits potential, several European-funded pilot projects, under science-business partnerships, have been dedicated to the assessment of the potential of algae exploitation in the biofuels and bioenergy sectors. Despite the

extensive research and investments in the last decade(s), no large-scale, commercial algae-to-biofuels facilities have yet been implemented. In fact, from existing algal cultivation sites, the produced biomass is currently exploited for production of food and feed, combined with the extraction of high added-value products, such as proteins, nutritional supplements and chemicals [21, 23].

Recently, a number of studies, mostly based on pilot/bench-scale plants or modelling of full-scale plants, presented several assessments of the algae-based biofuel production with respect to: environmental impacts, mainly life cycle GHG emissions, materials and energy balance and technological options and infrastructures, e.g. [24-26].

As for any type of biomass feedstock, the production of algal biofuels mainly depends on the properties of the selected algal species and particularly on those components that are being converted to biofuels. The most anticipated algal biofuel products include biodiesel from the conversion of the algal oils (i.e. lipids) that are extracted via chemical processes. Biogas can be produced via bacterial anaerobic digestion of the whole algal biomass or of the residues produced from oil extraction step. Bioethanol and biobutanol can also be produced by fermentation of the carbohydrates in algae by microbes or yeasts. Furthermore, hydrocarbon biofuels for heat, electricity and/or transportation fuels production can be obtained via thermochemical conversion of algal biomass at high pressures and temperatures [12, 25, 27, 28].

Considering the versatility of algae in biofuels production, a comparative assessment is necessary for identifying the most promising pathways, while highlighting the potential hot spots of the overall processing chain with respects to the energy balance and environmental impacts. Also, based on the limited current algal biofuels applications, the potential benefits of alternative technologies and approaches for co-products management need to be better understood, from a technological and environmental perspective.

1.2 Scope and methodology

The scope of this work is to report on the current status and development in the potential exploitation of algae (both macro- and microalgae species) as a feedstock for biofuels production. To this aim, the work presents a comprehensive review of the most promising algal biofuel pathways, based on recent findings and developments, in terms of technological options, opportunities and limitations to their overall effectiveness. The work discusses two major categories of algae that are defined based on their size, namely:

- Microalgae: unicellular organisms, size of tens of micrometers;
- Macroalgae (or "seaweeds"): complex multicellular structures, size up to tens of meters.

The specific objectives of this review include the:

- assessment of the current status and perspectives of different macro- and microalgal strains, in terms of chemical composition and productivities under specific cultivation conditions (i.e. technology, resource supply and climatic conditions);
- investigation of the downstream processing, such as harvesting, drying and conversion technologies for production of algal biofuels and non-fuel commodities;
- identification of promising pathways that can provide favourable energy and GHG emissions balances, while recognizing the main hotspots, in terms of energy consumptions and GHG emissions.

The work also aims to collect data inventory from literature and present an overview of the published Life Cycle Assessment (LCA) results, especially in terms of energy expended and GHG emissions, while highlighting gaps and methodological assumptions. The LCA can be an effective tool in assessing the environmental performances of algal

biofuels production along all its life cycle. To this purpose, LCA studies include processes from the extraction of resources and energy supply to the delivery of the fuel. Such assessments allow a comparative evaluation of the prominent biofuels pathways and co-products from macroalgae and microalgae.

A comparison of the performance associated to the proposed algal biofuels production pathways with that found for conventional fossil is also considered.

It should be noted that, over the last decades, the majority of algal biofuels research focused on the use of microalgal species, mainly for biodiesel production, while less efforts involved the use of macroalgal species. In fact, the potential of biofuels from microalgae is discussed in a number of studies based on the implementation of various existing pilot/bench-scale plants and projected development scenarios. On the other hand, biofuels production for macro-algae is less documented in the scientific literature. This is due to the fact that there is less interest and maybe less potential for intense macroalgal production.

An overview of the main stages of biofuels production from macroalgae (or seaweeds) and microalgae is found in Table 1.1. These processes will be described in next chapters 2, 3 and 4, in terms of technology options, energy requirements and GHG emissions. To this purpose, we have collected up-to-date experimental, theoretical and projected data from literature to produce a coherent set of possible algae-based biofuels (and co-products) pathways. Furthermore, we have identified the needed materials and potential energy in/out flows of the main steps of each considered algae-to-biofuels scenario. Literature included in this review consists of scientific articles published in peer-reviewed journals and technical reports. We have identified a number of articles issued in scientific journals and technical reports published between 2009 and 2015.

Table 1.1. Overview of the main process stages for production of biofuels from macroalgae and microalgae.

Process step	Macroalgae (or seaweeds)	Microalgae
Cultivation	natural stocks, drift material cultivation (near-shore systems, off-shore systems, open ponds)	cultivation (photobioreactors, open ponds)
Harvesting	manual mechanised	flocculation flotation sedimentation centrifugation filtration
De-watering/Pre-treatment	cleaning/washing crushing maceration	dewatering drying
Conversion to biofuels	biochemical processes: anaerobic digestion (AD) fermentation	biochemical processes: AD fermentation thermochemical processes: gasification hydrothermal liquefaction pyrolysis direct combustion trans-esterification and biodiesel production

1.3 Report structure

To give an overview of the most commonly macro- and microalgal species investigated, as well as an effective assessment of the process steps that are required for production of biofuels, the report structure has been set out in the following chapters:

- Chapter 2: addresses the main characteristics and composition of various algal species; the main features of algal cultivation systems under different growth conditions; harvesting and concentration techniques; current status and perspectives of algae-based fuels and products applications;
- Chapter 3: gives a general overview of the algae-based conversion pathways for the production of various bioenergy and/or biofuels options, such as biodiesel, biogas and bioethanol, among others;
- Chapter 4: highlights the main features, assumptions, modelling approaches and results of LCA studies of algal biofuel pathways available in the literature, namely:

  i) Microalgal biodiesel pathways via chemical processes;

  ii) Microalgal biocrude pathways via thermochemical processes;

  iii) Macroalgal biogas and bioethanol pathways via biochemical processes;

- Chapter 5: presents the overall conclusions and highlights the main constraints for commercialization potential of algae for biofuels applications. The main challenges and priorities for future work are also highlighted.

2 Algae species

2.1 Background

The term "algae" refers to a highly diverse group of eukaryotic organisms, mostly containing chlorophyll, which are either cultivated or wild harvested, originating from various aquatic environments. Algae are recognised as one of the oldest life-forms. Between 40,000 and 100,000 species of algae have been identified so far, though that number might even underestimate the actual number [23, 29, 30].

Most algae, like terrestrial plants, grow as photoautotrophs, being able to fix inorganic carbon from atmospheric CO_2 and to convert sunlight into chemical energy via photosynthesis ($6CO_2 + 6H_2O$ + light energy $\rightarrow C_6H_{12}O_6$ (sugars) + $6O_2$). The sugars formed by photosynthesis are then converted to other cellular components, such as lipids, carbohydrates and proteins, that make up the biomass matter [12].

In contrast, certain algae are heterotrophs, utilizing an organic carbon substrate (mainly glucose, acetate and fructose among others) as the only carbon and energy source, while turning it into chemical energy. This process implies the use of fermenters that are supplied by oxygen for the algal metabolic growth (also known as aerobic respiration) under dark conditions.

Furthermore, some species are mixotrophic, as they can simultaneously conduct phototrophic and heterotrophic processes to accumulate energy for growth, while consuming both inorganic CO_2 and organic carbon substrates [12]. Certain species (such as blue-green microalga *Spirulina*) experience maximum growth rate in mixotrophic culture, simultaneously using light and glucose, compared to photoautotrophic and heterotrophic cultures [31]. A more detailed description of algal biomass cultivation technologies under different growth conditions, i.e. photoautothrophic, heterotrophic and mixothrophic environment, is provided in section 2.6.

Overall, algae can be classified into two major groups based on their size, namely:

- Macroalgae (or seaweeds): consisting of multicellular organisms growing from 50 centimetres up to 60 meters in length. They are typically made of a blade or lamina, anchoring their entire structure to hard substrates in marine environments. Their growth cycles are complex and diverse, with different species displaying variations of annual and perennial life histories, combinations of reproductive strategies and alternation of generations [12, 32];
- Microalgae: comprising unicellular organisms varying from nano- to milli- meters in size. They can be found in a variety of aquatic habitats, being able to thrive in freshwater, brackish, marine and hypersaline aquatic environments. They have been identified also in desert crust areas, thereby being able to endure extreme temperature and low water availability [12, 33].

Macroalgal and microalgal groups present different morphological, structural and chemical features, as well as evolution pathways. The following sections describe the main characteristics of the macro- and microalgal species, as well as their cultivation systems.

2.2 Macroalgae (or seaweeds)

2.2.1 Characteristics and composition

Macroalgae (or seaweeds) are multicellular plants growing in salt or fresh water. They are often fast growing and can reach sizes of up to 60 m in length [34].

Macroalgal species primarily occur in near-shore marine coastal waters, where they grow attached to rocks or suitable substrates. In these conditions, some species can form stable, multi-layered and perennial vegetation. These organisms have been recognized as essential components for preserving the biodiversity of marine ecosystems [23, 35].

In addition, macroalgae can be found in the open ocean, sea and freshwater habitats as floating forms [36]. Another relevant source of seaweeds includes the so called "drift seaweeds" that tend to develop along the coastal areas and in shallow estuaries or bays due to eutrophication, i.e. the enrichment of surface waters with nutrients, such as nitrogen and phosphorous from agriculture and sewage outfalls [37]. These phenomena are also known as "green tides" that are commonly dominated by the presence of drift *Ulva*. The location and seasonal availability of the green tides is hard to predict. Some studies reported that the occurrence of drift *Ulva* has an increasing trend along the European coasts of Ireland, Denmark, and France [23, 37]. Drift *Ulva* products are considered as waste and they are not yet used for industrial applications. However, it is suggested that this source of biomass provides an opportunity for biomethane production through AD [37].

The growth rates of macroalgae far exceed those of terrestrial plants. On average, productivities of approximately 3 to 11.3 kg (dry wt.)/m^2 per year for non-cultured algae and up to 13 kg (dry wt.)/m^2 per year for cultured algae have been measured for six selected species [38]. In comparison, average productivities from 6 to 9.5 kg (wet wt.)/m^2 per year are found for sugar cane, that is one of the most productive land plants [35, 39]. In addition, marine biomass does not require fertilisation as the water movement provides a continuous flow of a base level of nutrients, such as nitrates and phosphates [40].

Based on the composition of their photosynthetic pigments, macroalgae are classified into green (*Chlorophyceae*), red (*Rhodophyceae*) and brown (*Phaeophyaceae*) species, giving diverse cellular structures and evolution pathways.

Depending on the species and growing site location, macroalgae contain different proportions of lipids, proteins and carbohydrates. The major chemical composition of selected green, red and brown macroalgae of marine and freshwater origin, which were evaluated during experimental campaigns, are shown in Figure 2.1 [36, 38, 41]. The results indicate that carbohydrates are the main organic constituent of most species, ranging from 23 to 79.4 % dry wt.. Furthermore, seaweeds are characterised by low lipids, with content of less than 10% dry wt. for all the analysed species. As for the proteins, the results in Figure 2.1 indicate that the amounts vary from 6.9 to 28% dry wt.. The ash content is typically in the range 4.4-63.9 % dry wt., depending on the species. Overall, the results indicate that higher ash contents are found for the red and brown species compared to the green ones, with average values of 30 to 60% (excluding the species *Gelidium Amansii*, for which the lowest value of about 4% of ash is found) [36]. It is evident from the literature that seaweeds have low cellulose and zero lignin contents [42]. A seasonal variation in the chemical composition of macroalgae can be observed, in relation to environmental factors of the growth ecosystem, such as the light intensity, temperature, nutrients and CO_2 availability [40]. The variation of carbohydrates in macroalgae can be significant depending on the season. For instance, variations between 17 and 23% dry wt. in *Ulva* in the period of June-September and between 5 and 32% dry wt. for *Laminaria digitata* over a whole year are reported in [43]. Overall, the maximum carbohydrates content is found in autumn. In contrast, during the winter season, the stored carbohydrates are utilized as energy source for the synthesis of proteins, as well as reproduction and growth. Macroalgal species, which are able to accumulate high levels of carbohydrates, are considered suitable for production of biomethane, bioethanol and biobutanol via microbiological conversion processes. Alternatively, these species can be exploited for production of chemicals with an attractive high economic value [43]. Detailed descriptions of cultivation and process systems for producing macroalgae-based biofuels will be provided in the next sections.

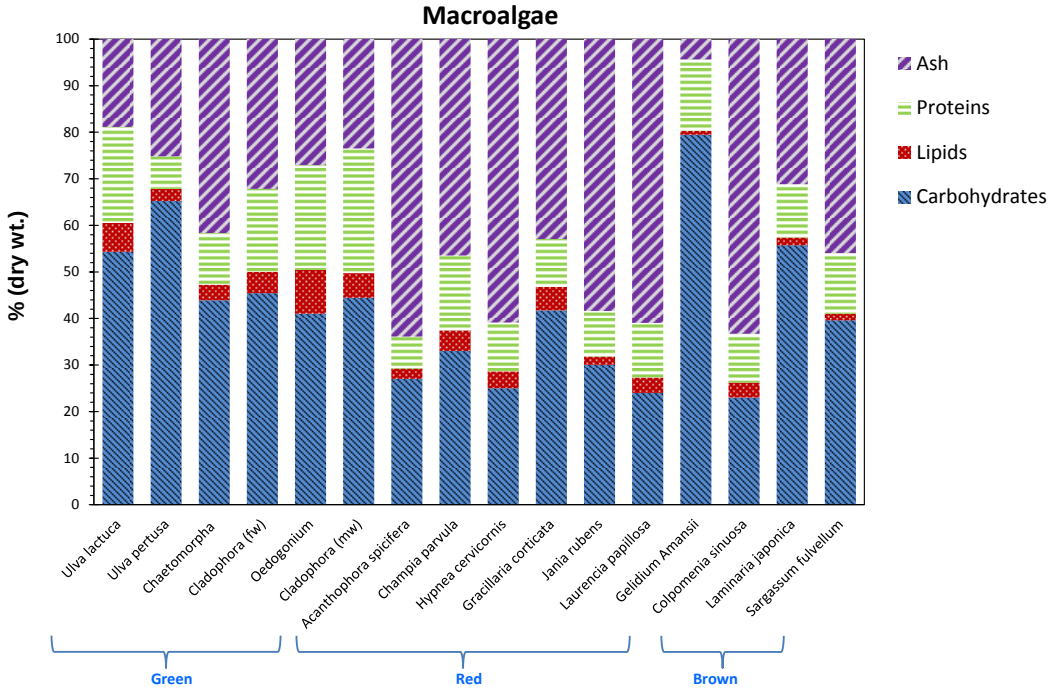

Figure 2.1. Chemical composition of various green, red and brown macroalgal species, [36, 38, 41]. Results are expressed as percentage of dry wt. biomass.

2.2.2 Cultivation

In 2011, the global amount of cultivated macroalgae accounted for about 15 million (wet wt.) tonnes, as shown in Figure 2.2 [36]. The majority of seaweeds are cultivated in Asian countries (75% of global production occurs in China) mainly to produce food and hydrocolloids for the food, pharmaceutical and chemical industries. Almost all (99%) of cultivated species belong to the red and brown groups, as they can be used in different industries [44]. Figure 2.3 displays examples of commercially exploited red macroalgae [35].

In Asian countries, macroalgal farming in commercial cultivation sites has been commonly practiced since the 1970s. Recent research has shown the potential of large-scale macroalgal cultivation also in the Atlantic waters of Canada [39, 45].

In Europe, cultivation of macroalgae is currently at an early stage of development, while the majority of the available resources are manually or mechanically harvested from wild stocks (e.g. Acathophora spicifera, in inset b of Figure 2.3 [36]). In 2011, the estimates of harvested seaweeds corresponded to around 23,000 wet wt. tonnes in the Irish and Scottish coastal areas [39, 46]. Nevertheless, many concerns exist about the high exploitation of wild macroalgal stocks, considering the importance of these species in preserving the biodiversity of marine habitats for wide ranges of organisms (e.g. fish, birds and seagrasses). Furthermore, wild macroalgae are generally dispersed around the coastlines, often implying high costs of harvesting and transport to processing plants [45]. Hence, a deeper understanding of the environmental impacts of harvesting seems necessary before setting up strategies leading to massive exploitation of wild macroalgae.

On the other hand, macroalgal farming is required to generate significant volumes of biomass for potential biofuels supply [23]. Seaweeds production needs suitable aquatic environment and site location with appropriate temperature, light, nutrients and salt content, as well as water movement. Different farming systems have been developed worldwide, mainly including three options, namely offshore, near-shore and land-based facilities [23, 32, 47].

In offshore systems, seaweeds are typically grown attached to dedicated growth structures, with marine water as nutrients source. The seaweeds need supporting structures, like anchored lines/netting to be protected from possible intense swell and currents. These systems generally consist of 150-m long culture ropes which are anchored to 10 meters long structural ropes, as reported in [48]. The entire ropes systems are maintained 2 meters below the water surface, anchored to the bottom of the sea by means of concrete blocks. The foundations of off-shore wind farms can also be used as anchoring points for the supporting structures. Modern cultivation technologies for green macroalgae (*Laminaria hyperborean*) have been successfully tested in the North Sea [46, 47, 49]. Though, some difficulties were encountered with the stability of the structures themselves or the attachment of macroalgae to the growth structures [32]. Furthermore, risks of losses and colonisation by other organisms have been identified for some species [47]. Additional research on the system design and techniques for macroalgal harvesting and processing in large off shore farms is needed in future works.

Near-shore systems in coastal environments, such as river estuaries, require macroalgal farming devices that are similar to those used in offshore farms. Near-shore cultivation of macroalgae is currently commercial in Asian countries. In contrast, in the United States and Europe, environmental regulations and social resistance provide major challenges to the use of coastal zones for developing large-scale cultivation of macroalgae [32, 49]. On the positive side, it should be noted that these farms can be used as bio-filters, being able to remove nitrates and phosphate from the surrounding waters during the growth phase [39]. Projected estimates of brown macroalgal species (*Saccharina latissima*) growing in 100 m long lines in Scottish sea lagoons correspond to about 200 tonnes wet wt./ha [45]. In addition, pilot studies indicated that yields of 15-20 tonnes dry wt./ha can be achieved for four species (*Laminaria Digitata, Saccharina Latissima, Palmaria palmata* and *Ulva Lactuca*) cultivated in the North Sea [47].

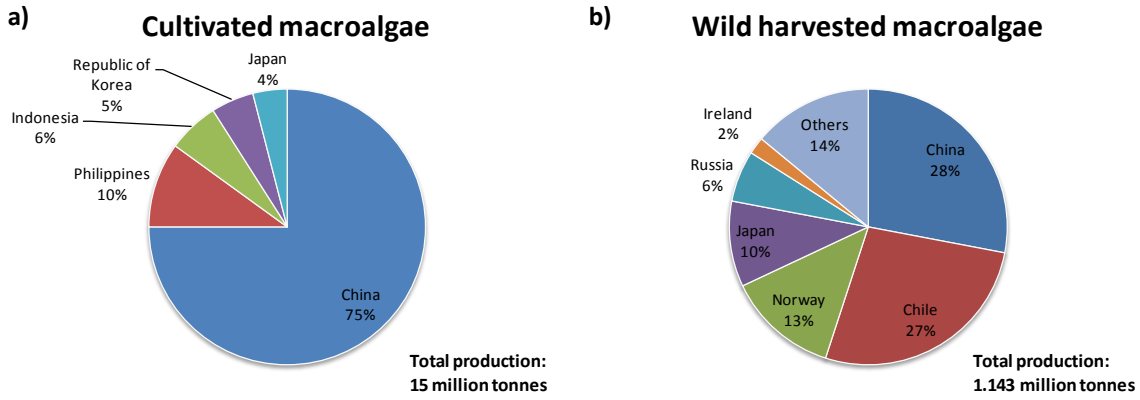

Figure 2.2. Annual estimates of cultivated (inset a) and wild harvested (inset b) macroalgae by countries worldwide in 2011 [36]. Results are expressed in wet wt. tonnes.

Land-based pond systems have also been considered for macroalgal cultivation, both as free standing farms or in combination with land-based aquaculture systems of e.g. molluscs and other fish culture [32]. These systems can avoid the fluctuating conditions occurring at open sea site, such as changes in temperature, salinity, currents (tidal actions), disease and predation that can affect the possible macroalgal yield [45]. However, major efforts to scale-up current activities are needed to provide affordable biomass supply for biofuels production [39].

Additional considerations on the energy balance and GHG emissions of macroalgal cultivation for biofuels production can be found in chapter 4.

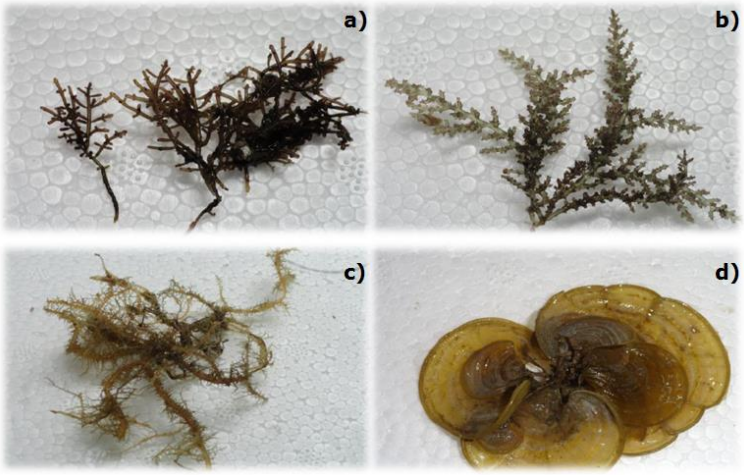

Figure 2.3. Examples of commercially available macroalgae, such as: -inset a: *Graciliaria dura* (red species); -inset b: *Acathophora spicifera* (red species); - inset c: *Hypnea esperi* (red species); -inset d: *Padina pavonica* (brown species) [35].

2.2.3 Harvesting and concentration

Little information is currently available on the harvesting process of both wild and cultivated seaweeds due to the limited development of seaweeds-based products. It is reported that when seaweeds species have reached a mature stage, they are harvested by either leaving a small piece that will re-grow afterwards, or removing the entire plant and cutting small pieces for further cultivation [32, 46]. Manual harvesting of seaweed (for food uses) has been practiced for centuries and it is still common for species naturally growing in coastal areas [47]. On the other hand, mechanical harvesting is required for collecting large quantities of wild or cultivated macroalgal stocks. The type of mechanized systems mainly depends on the form and growth characteristics of macroalgae. The most common systems include: -rotating blades that can be suitable for species growing attached to supporting structures; and -suction systems followed by cutting that can be used for floating seaweeds species (e.g. *Sargassum* and *Gracilaria*) [46]. Mechanized harvesting systems require floating vessels for operation. Furthermore, modern vessels can be equipped with pumps for harvesting macroalgae into a net or other containment structures [32]. Further development and evaluation of harvesting systems are needed from the technical and economical points of view to supply economically viable feedstocks for biofuel production.

Following the harvesting stage, macroalgae generally require pre-treatments to remove possible foreign objects, such as stones, sand or other debris that may have been caught in the biomass. These operations can be conducted either manually or by washing with water. Then, chopping or milling may be required to increase the surface area/volume ratio for more efficient conversion of microalgae to biofuels. Finally, the water content of macroalgal biomass should be reduced from its initial level of 80-85% to 20-30% to avoid degradation during storage, while reducing the costs of transportation to the processing plants [23, 32, 46]. An overview of the main practices required for seaweeds harvesting and processing before biofuels and/or bioproducts applications is found in Figure 2.4. In this respect, it should be considered that proper assessments of materials and energy inputs, as well as cost-effectiveness are necessary to evaluate best practices for wild/cultivated macroalgae for biofuels and bioproducts production. Nevertheless, to the authors' knowledge, there is no available publication detailing these practices. Literature information indicates that waste heat from coal-fired boilers is often used for drying seaweeds [10]. Furthermore, experimental solar drying configurations have been set up in a recent project [50]. The systems could achieve the final water content of 10% starting from 90% (on a wet basis) in 15 hours, under the solar radiation of about 500 W/m^2 and air flow rate of 0.05 kg/s. The energy consumption of the system accounted for 2.62 kWh/kg of seaweeds [50]. The main disadvantages of sun drying

include the requirement for large drying surfaces and the risk of dry matter losses [11]. Nevertheless, severe drying treatment of seaweeds should be avoided to avoid high energy consumption of the overall macroalgae-to-biofuels systems. For that reason, fermentation processes for production of biogas, bioethanol and biobutanol would be preferable for macroalgae as they require a certain amount of water to operate efficiently.

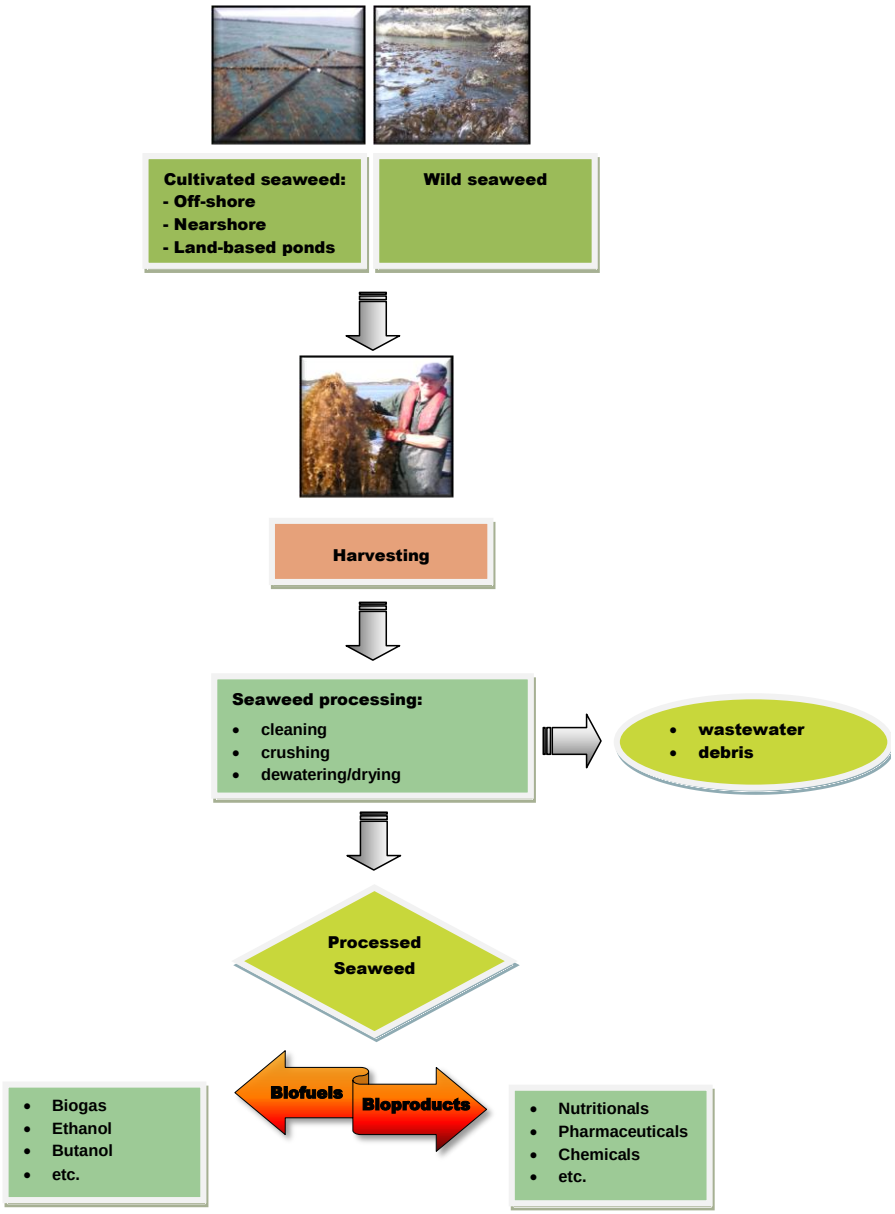

Figure 2.4. Main practices required for seaweeds cultivation, harvesting and processing before the implementation of biofuels and/or bioproducts applications, adapted from [10].

2.2.4 Current applications and future perspectives

Worldwide growth of research, technological development and patents registration have been identified for macroalgal cultivation systems in different countries between 1980 and 2009 [51]. As for the registered patents, South Korea and China experienced the

most rapid increase over the past 10 years (+28% and +20% per year, respectively), while Europe had a modest increase of 3.9% per year during the last decade. European commercial farming operations, notably in France, Germany and Ireland, are still at an early stage of development. Seaweeds are receiving increasing attention as potential renewable feedstock for production of gaseous and liquid transportation biofuels, such as biomethane and bioethanol.

According to the literature, the yields of wet biomass on long lines offshore systems typically vary between 6.2-11.7 kg/m after four months cultivation during spring time [48, 52]. These values largely depend on the selected species, time of planting (e.g. seasonal variations) and environmental conditions (nutrients availability). A recently published study evaluates the productivity potential of six selected macroalgal species in land-based cultivation systems, consisting of outdoor batch-cultivation tanks of 50 liters capacity [38]. Culturing tanks were supplied with the same regime of nutrients and water exchange. The results showed that the highest productivities were measured for marine macroalgae species (i.e. *Derbesia* and *Ulva*), corresponding to 11.9 and 11.4 g/m^2 (after a cultivation cycle of 6 days), respectively. Obviously, the growth rate and productivities of such systems need to be further assessed at larger scale and over a longer cycle, under continuous cultivation mode.

Major challenges for large-scale seaweed cultivation systems include the development of cost-effective methodologies to grow, e.g. off-shore farms/land-based pond construction and culture mixing, harvest and transport large quantities of biomass [39, 45]. Also, the potential benefits of seaweeds cultivation as an effective bio-filter to mitigate the enrichment of nutrients in coastal waters should be further assessed, in terms of cost-effectiveness and area requirements.

The study of [47] on seaweed production and applications in the North Sea indicates that, for the time being, there is no fully developed value-chain for macroalgae-based biofuels production. This is mainly related to the high production costs, which were estimated between 121 and 409 € per tonne of dry wt. macroalgae grown in long-line systems, excluding the operational, capital and labour costs [47]. Technical breakthroughs of macroalgal farming systems enabling multiple harvests per year may contribute to reduce the production costs [47]. Furthermore, the low revenues from macroalgal biofuels, such as biogas and/or bioethanol, make the economic feasibility of biofuels-only production quite low. Instead, producing multiple high-value products from macroalgae, such as hydrocolloids for the food industry, feed and chemicals, by implementing a biorefinery system is considered necessary to match the biomass production costs and to develop marketable products [53].

2.3 Microalgae

2.3.1 Characteristics and composition

Microalgae are unicellular organisms ranging in size from nano- to milli-meters, depending on the species, having chlorophyll as their primary photosynthetic pigment. They are found in aquatic habitats of marine and freshwater and also on the surface of all type of soils [11, 32]. Although they are generally free-living, certain microalgal species live in symbiotic association with a variety of other organisms [54].

Microalgae are mainly photosynthetic organisms, although various species can grow under heterotrophic conditions in the absence of light (see next section for further information on microalgae cultivated via photoautotrophic or heterotrophic methods) [32, 54]. Comparing to macroalgae and land plants, microalgae are generally more efficient converters of solar radiation into usable energy via photosynthesis due to their simple cellular structure. Microalgae are characterized by generation times that are usually higher than 24 h, although some strains are able to duplicate their cells in less than 8 hours [12]. This is mainly because they have more efficient access to water, CO_2 and nutrients during photosynthetic growth [10, 12, 55-58].

Microalgae can occur in natural stocks, such as blooming of marine populations in lakes, ponds or open seawater. As for seaweeds, large and harmful microalgal blooms may also result from water eutrophication. In these conditions, the excess of microalgal biomass undergoes microbial degradation, reducing the oxygen levels in the water. Decreases in the oxygen levels below acceptable limits may have negative impacts on the ecosystem leading to e.g. fish mortality. It is, hence, believed that collecting microalgal blooms would be beneficial for avoiding negative impacts on the ecosystem, while providing an opportunity for their exploitation as biomass sources [23].

Classification of microalgae is based on chemical and morphological characteristics. Major groups of microalgae can be identified mainly depending on their pigments composition, cells structure, chemical constituents and life-cycle, namely: - green algae (*Chlorophyceae*); - blue-green algae or cyanobacteria (*Cyanophyceae*); - golden-brown algae (*Chrysophyceae*); - diatoms (*Bacillariophyceae*); and *eustigmatophyceae*. Detailed description of microalgae biology and classification is reported in previous works [12, 22, 56, 59]. Figure 2.5 shows examples of green microalgae investigated in "Miracles-specialties from algae" that is an ongoing project funded under the European Union's seventh framework programme for research, technological development and demonstration.

Figure 2.5. Examples of green microalgal strains being investigated in "Miracles-specialties from algae" that is an ongoing project funded under the European Union's seventh framework programme for research, technological development and demonstration. Pictures from publications available on the project website (http://miraclesproject.eu/).

Since the 1920s, a number of marine and freshwater microalgal strains have been investigated by research institutes and Universities in different countries, such as USA, Japan, Australia, Portugal and Germany [60]. An overview of the major chemical components of various microalgae that can be found in the literature is provided in Figure 2.6.

Overall, the results indicate that the proteins are the main components of most species under consideration, with concentrations varying between 30-71% for almost all the species shown in Figure 2.6. The results also show significant variations in the carbohydrates concentrations, ranging from 4 to 58% of dry wt.. The highest carbohydrates concentrations of about 50-57% (dry wt.) were measured for selected species of the green microalgal group.

With regard to lipids, results indicate variations in concentrations depending on the considered species. High concentrations above 35% and up to 45% of dry wt. were found for a few selected species shown in Figure 2.6 (see *Scenedesmus dimorphous; Orymnesium parvum* and *Nannochloropsis sp.*). On the other hand, lipids concentrations in most species are found between 2 and 19% of dry wt.

Overall, the chemical composition of microalgae significantly varies among the different species and throughout the year, depending on environmental factors, such as the light intensity and temperature. Furthermore, composition of microalgae is highly dependent on the (indoor/outdoor) growth conditions and nutrients availability [12, 59]. For instance, the lipids content of selected species can be increased from 17.5 to 38.5% dry wt. biomass when microalgae are cultivated under nutrients starvation, i.e. low Nitrogen and Phosphorus supplies. The composition of microalgal species grown under Nitrogen-sufficient and Nitrogen-starvation conditions is shown in Figure 2.7. Further details on the energy and materials supplies for cultivation of microalgae can be found in chapter 4 [12, 24, 61].

Extensive results, which were mainly derived from experimental laboratory or small-scale tests, on the lipids content (as % of dry wt.) and productivity (in mg/l/day) of various microalgae strains are found in the literature, as indicated in Table 2.1. This is mainly because lipids (including triacylglycerols, TAG) represent one of the main components of microalgae that could be extracted and converted into liquid biofuels products, mainly biodiesel [12, 21, 62]. Possible microalgal strains of different groups, such as green, eustigmatophyceae and diatoms, that can be promising for biodiesel production pathways are shown in Table 2.1.

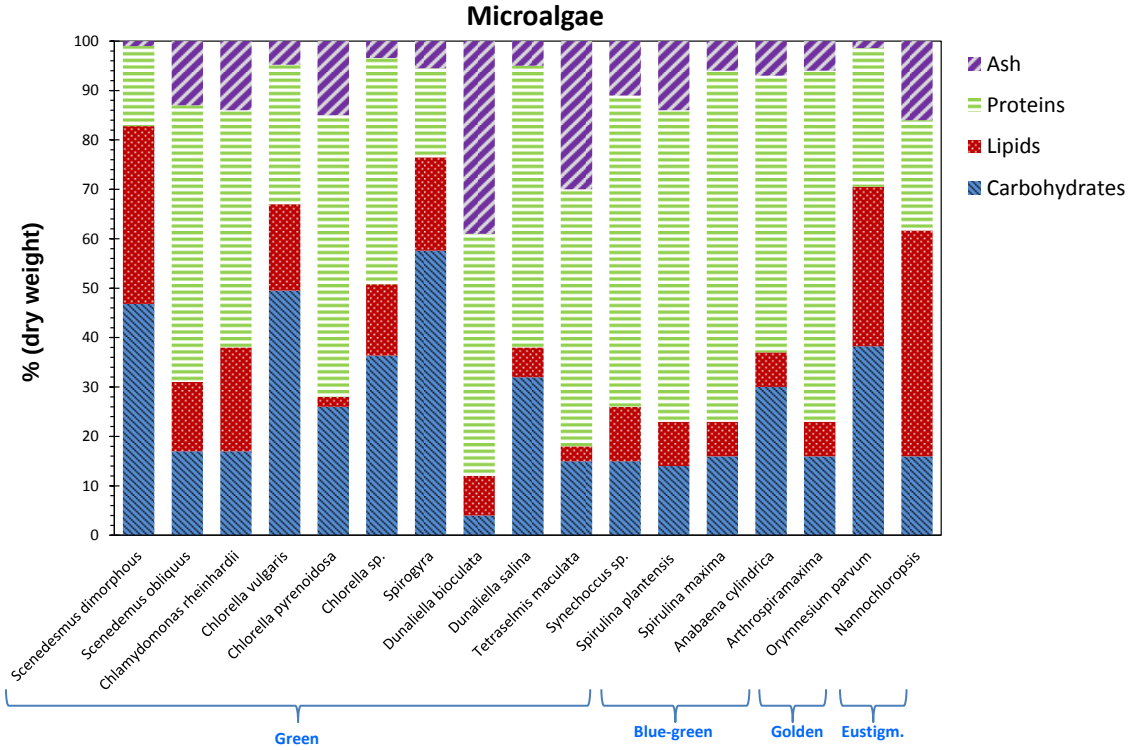

Figure 2.6. Chemical composition of microalgal species of different groups (green, blue-green, golden and eustigmatophyceae), from [63, 64]. Results are expressed as percentage of dry wt. biomass.

The lipids content of microalgae grown under different environmental conditions was found to significantly vary depending on the species. Overall, many species present lipids content ranging between 15-60% of dry wt.. On the other hand, lipids levels lower than 15-10% of dry wt. biomass can be observed for some species reported in Table 2.1. The biggest range in concentrations is observed for *Chlorella vulgaris* (green specie) with possible lipids values ranging from 5 to 58% of dry wt. The differences in lipids concentrations for each species considered depend on the combination of different factors relating to the culture media, e.g. water, nutrients concentration, cultivation methods (operation in batch or continuous or semi-continuous mode) and technology (open or enclosed systems) [65, 66].

As for the lipids productivity, it can be observed that microalgae of specific groups, i.e. green and eustigmatophyceae, are characterized by a higher lipids growth rate (maximum estimates of 1,214 mg/l/day) than diatom groups. Similarly to the lipids contents, many factors may affect the lipids productivity of the various microalgae species, including the culture media, cultivation methods and environmental conditions, e.g. pH ,temperature, salinity and light intensity [65, 66].

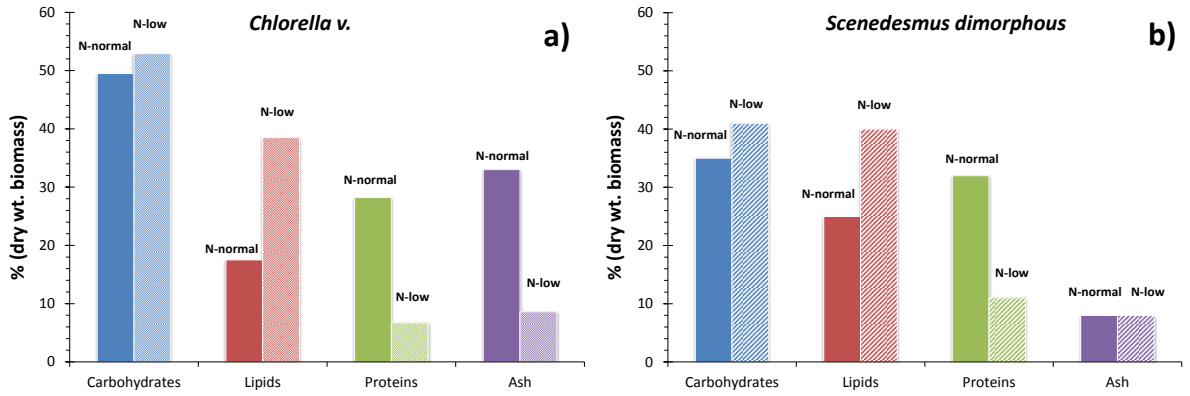

Figure 2.7. Concentration of carbohydrates, proteins, lipids and ash (% dry wt.) of selected microalgal strains, i.e. *Chlorella v.*, inset a, and *Scenedesmus d.*, inset b, grown under sufficient N and low N supplies in ORP [24, 67].

Commercial scale cultures of microalgae are well-established in Asia, United States (US), Israel and Australia since the 1980s [18, 22]. Currently, about 9,200 dry wt. tonnes of microalgae are annually produced worldwide mainly for dietary or health food for human consumption and feed additives in aquaculture [44, 59, 68]. Figure 2.8 shows the amounts of mass-cultivated species in different countries (only aggregated values for various countries can be found in the literature). The most abundant strains correspond to *Arthrospira plantensis* and *Haematococcus pluvialis* with production of about 3,000 dry wt. tonnes each, being cultivated in Asia, US and Israel. In comparison, other species like *Chlorella sp.* and *Dunaliella salina* are produced in smaller amounts, accounting for 2,000 and 1,200 (dry wt.) tonnes, respectively. Production of the former two species mainly occurs in Asia and Israel, but also in Europe (Germany, cultivation of Chlorella) [59].

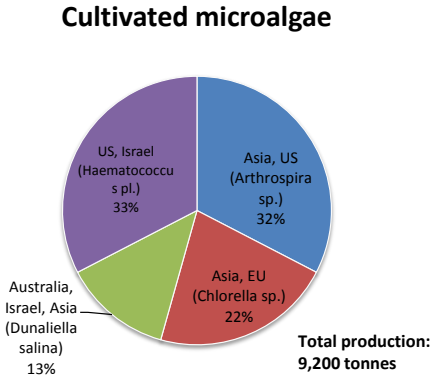

Figure 2.8. Annual estimates (in dry wt. tonnes) of cultivated microalgae for food and feed production worldwide, (from [44, 59]).

While there is a well-established global market for microalgae-based food and feed products, microalgae-based biofuels applications have not yet been commercially developed [21, 68]. As mentioned above, the cultivation of microalgae is currently limited to the production of highly valuable molecules, such as proteins, polyunsaturated

fatty acids (PUFAs) and pigments, such as carotenoids and astaxanthin, with high commercial values [21, 60].

The majority of microalgal commercial production is carried out in large open ponds or lagoons [59]. Instead, commercial production in photobioreactors (PBR) is limited to a few hundred tonnes. This is mainly due to the fact that open ponds are easier to operate, less expensive and more durable than closed PBR [59, 61]. A detailed overview of the existing processes and technologies for microalgae cultivation and extraction of biofuel precursors will be given the next section 2.3.2 and chapter 3.

Table 2.1. Lipids content (% dry wt. biomass) and productivity (in mg/l/day) of various microalgal strains [56, 65].

Microalgal strains	Lipids content	Lipids productivity
	% dry wt. biomass	mg/l/day
Green		
Chlorella emersonii	*25-63*	*10.3-50*
Chlorella protothecoides	*14.6-57.8*	*1,214*
Chlorella sorokiniana	*19-22*	*44.7*
Chlorella vulgaris CCAP 211/11b	*19.2*	*170*
Chlorella vulgaris	*5-58*	*11.2-40*
Chlorella sp.	*10-48*	*42.1*
Chlorococcum sp. UMACC 112	*19.3*	*53.7*
Dunaliella salina	*16-44*	*46.0*
Nannochloropsis oculata NCTU-3	*30.8-50.4*	*142*
Nannochloropsis oculata	*22.7-29.7*	*84-142*
Neochloris oleoabundans	*29-65*	*90-134*
Scenedesmus quadricauda	*1.9-18.4*	*35.1*
Schizochytrium sp.	*50-57*	*35.1*
Tetraselmis suecica	*8.5-23*	*27-36.4*
Tetraselmis sp.	*12.6-14.7*	*43.4*
Diatoms		
Chaetoceros muelleri	*33.6*	*21.8*
Chaetoceros calcitrans	*14.6-39.8*	*17.6*
Phaeodactylum tricornutum	*18-57*	*44.8*
Skeletonema sp.	*13.3-31.8*	*27.3*
Skeletonema costatum	*13.5-51.3*	*17.4*
Thalassiosira pseudonana	*20.6*	*17.4*
Eustigmatophyceae		
Ellipsoidion sp.	*27.4*	*47.3*
Nannochloris sp.	*20-56*	*60.9-76.5*

2.3.2 Cultivation

As previously discussed for macroalgae, dedicated production systems are required to generate significant volumes of microalgae to be exploited in the biofuels and bioenergy sector. Microalgae can be cultivated mainly using three methods, under different nutrients supply [12, 60, 69], namely:

- phototrophic cultivation: microalgae make use of light as energy source and CO_2 as inorganic carbon source for their photosynthetic growth;
- heterotrophic cultivation: microalgae grow without light, i.e. in a dark environment, utilizing organic substrate, such as glucose, acetate and glycerol as both energy and carbon source;
- mixotrophic cultivation: microalgae are able to grow either via phototrophic or heterotrophic conditions, depending on the concentration of organic carbon sources and light intensity.

Some microalgal strains (such as *Chlorella vulgaris, Haematococcus pluvialis, Arthrospira platensis*) have been found to grow under photoautotrophic, heterotrophic, as well as mixotrophic conditions [32, 60, 66]. Phototrophic microalgae offer the main advantage

to capture CO_2 streams from flue gases. However, this method has major limitations in locations where proper sunlight intensity is not always available throughout the year [66, 70]. On the other hand, heterotrophic cultures overcome this problem as microalgal strains can grow in a dark environment, while still attaining high lipids yield and biomass productivity. Nevertheless, heterotrophic systems present significant issues to be taken into account, such as: i) high risks of contamination by other microorganism due to the presence of organic substrates as carbon sources; ii) high energy requirements and costs of the upstream supply [70]. Therefore, this cultivation method is regarded as not-promising for a viable algal biofuels production chain.

In addition, it should be considered that, for each cultivation methods, specific operational inputs (like nutrients; vitamins; salts, oxygen and carbon dioxide) and parameters (as pH; temperature and light intensity) are essential for optimal algal growth [12, 70]. Therefore, it is important to determine the interrelation of such operational parameters with the biomass growth, under the considered cultivation conditions.

As already mentioned, the most common cultivation systems used for photoautotrophic microalgal growth, i.e. in the presence of light, include: Open Raceway Pond (ORP) and PBR. The former category comprises natural lakes, lagoons and artificial ponds where algae are grown in suspension. Commercial microalgal production systems mainly use ORPs, consisting of an open shallow pond with an elliptical shape, where the water is mechanically mixed by means of a paddlewheel. In this design, algae absorb the sunlight and CO_2 from the air, while fertilizers (N, P and K) can be added to the water. The movement of the water along the raceway avoids the settlement of algal biomass, while stabilizing the growth and productivity of algae. The ORPs, which are usually maximum a third of an hectare in size, may be built in concrete or compacted earth and lined with plastic [32, 66]. Examples of ORP configurations that can be found in the literature are shown in Figure 2.9.

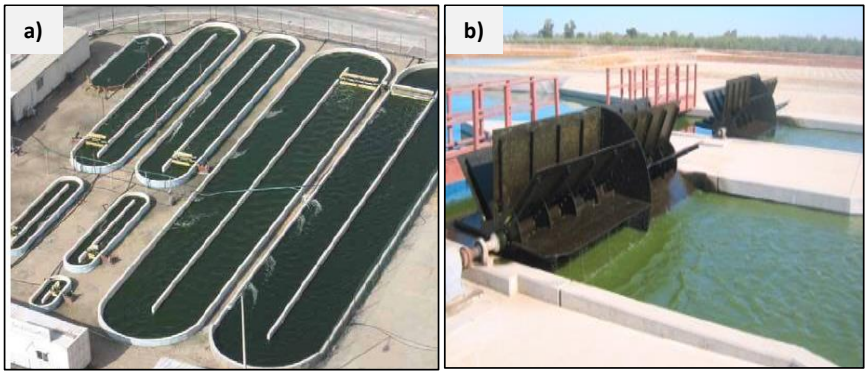

Figure 2.9. Examples of ORP systems used for production of microalgae. Inset a) refers to the commercial production of *Nannochloropsis sp.* in Israel [49]; inset b) refers to ORP systems located in California [14].

Production of microalgae biomass in ORP has been practiced since the 1950s in Japan, the US and Germany [11]. The major advantages of ORP are the ease of construction, operation and maintenance. On the other hand, the main drawbacks include: i) poor light utilization; ii) low yield of biomass; iii) evaporative losses and iv) high risks of contamination by algal predators or fast-growing microorganisms. Detailed descriptions and analyses of the available system configurations are reported in several published works [11, 22, 23, 49, 62, 66]. As mentioned above, ORP is the main system implemented at a commercial scale for cultivation of microalgae to produce human nutritional products (i.e. beta-carotene and astaxanthin from *Dunaliella salina* and

Haematococcus pluvialis, respectively) [68]. Furthermore, ORP systems can be used for the wastewater treatment along with the production of microalgal biomass [14, 16].

The algal biomass productivity that can be obtained in ORP ranges between 5-25 $g/m^2/day$, mainly depending on the: i) selected strain; ii) water and nutrients supply (e.g. nitrogen deprivation and CO_2 enrichment) and iii) local conditions, in terms of temperature and light intensity [14, 16, 71, 72]. Further insights on the biomass productivity in ORP systems can be found in chapter 4 focusing on the review of LCA studies.

Unlike the ORPs, PBRs are enclosed systems where the algal growth conditions can be continuously and precisely monitored. In addition, PBRs allow the culture of single-species of microalgae for prolonged durations with low risks of contamination. PBRs can be located outdoor utilizing sunlight or indoor utilizing artificial light [62].

Since the 1980s, many PBRs have been designed, including: i) flat plate; ii) annular and tubular systems. The different categories can be developed in horizontal, vertical or inclined configurations. Construction materials employed for PBRs are usually plastic or glass [59, 66, 72]. Among the different designs, the tubular PBR in vertical and horizontal configurations have been most commonly developed so far [59].

PBRs enable a rigorous process control and potentially much higher concentration of biomass and lipid productivity ($g/m^2/day$) than ORPs. Depending on the specific configurations and selected strain, the average biomass productivity in PBR ranges between 60-650 $g/m^2/day$, with the highest values obtained for flat plate systems [65, 72].

Furthermore, combination of two distinct algal growth stages in PBR and ORP has also been implemented through hybrid cultivation systems to optimize the lipids production of selected strains. The first stage is implemented in PBR systems to achieve high biomass growth under controlled conditions. The second stage is carried out in ORP systems to enhance the production of lipids under nitrogen deprivation conditions [61, 73, 74]. As identified in a previous study, selected hybrid systems could give annual lipid production rates of 20-30 tonne of oil equivalent per hectare, under favourable tropical climates [61].

The major disadvantages of PBR are the high capital costs for construction of the systems [75, 76]. Also, high operational costs are generally found for PBRs, due to the energy requirements for: i) cleaning of both the internal and external walls when fouling problems occur; ii) mixing of the culture to maintain turbulent flow through the system; iii) pumping and sparging of gases for algal growth; iv) nutrients and water supply [59, 75].

The modelling results of [75], which only include the algal cultivation and harvesting process steps, show that the production cost of algae in PBRs can be as much as 3.8 €/kg, which is higher than that found in ORPs corresponding to 0.3-0.4 €/kg. It should be noted that all the estimated costs consider that nutrients and water are supplied by wastewater and they are then free of charge. Furthermore, the results consider idealized conditions of microalgal growth, in terms of biomass productivity and energy efficiency. The projected costs most likely underestimate the costs of microalgal production under a possible real-scale scenario also because the analysis excludes the cost of finance and the cost of land [75].

A comparison of the main advantages and disadvantages of ORP and PBR systems is reported in Table 2.2. Nevertheless, comparison of performances achieved by PBR and ORP is not straightforward. In-depth evaluations of the two systems should take into account several factors, such as the selected algal strains for cultivation and site location. For the time being, there is no consensus about which cultivation method is preferable for microalgae production. Overall, the analysis of energy balances, GHG balances and costs is needed on a case-by-case basis. Improvements of the existing

algal production strategies are then required to achieve reduction of production costs, as well as development of stable and reliable cultures for both technologies [26, 75].

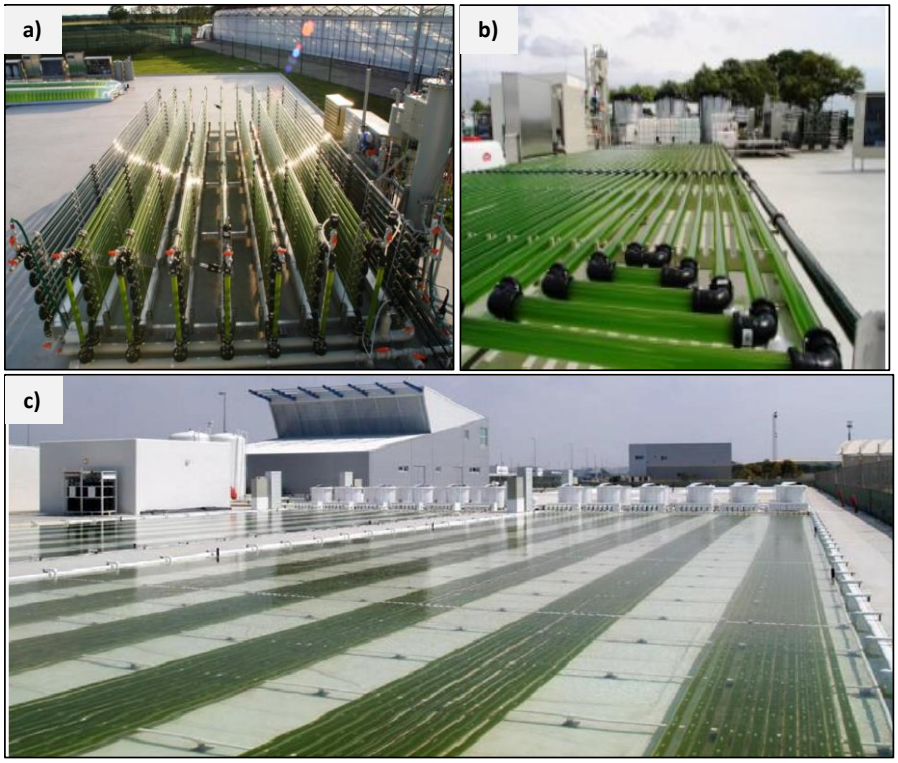

Figure 2.10. Examples of demonstration PBRs; the insets a) and b) show tubular PBR in vertical and horizontal configurations, respectively, as developed at Wageningen University (The Netherlands), from AlgaeParc website (http://www.wageningenur.nl/en/Expertise-Services/Facilities/AlgaePARC.htm). Inset c) shows tubular PBRs developed at Fitoplancton Marino SA (Spain), from "Miracles-specialties from algae" website (http://miraclesproject.eu/).

Table 2.2. Main advantages and limitations of open ponds and tubular/flat panel PBR systems [21, 60, 77].

Production system	Advantages	Limitations
ORP		
	Easy to clean	Poor biomass productivity
	Easy maintenance	Large area of land required
	Low energy inputs	Limited to a few strains of algae
		Poor mixing, poor light and CO_2 utilisation
	Good for mass cultivation	Contamination risks for algal cultures
	Relatively cheap	Difficulty in growing algal cultures for long periods
Tubular PBR		
	Large illumination surface area	Some degree of wall growth
	Suitable for outdoor cultures	Fouling
	Good biomass productivities	Requires large land area
		Gradients of pH, dissolved oxygen and CO2 along the tubes
Flat plate PBR		
	High biomass productivities	Scale-up require many compartments and support materials
	Easy to sterilise	Difficult temperature control
	Low oxygen build-up	Small degree of hydrodynamic stress
	Readily tempered	Some degree of wall growth
	Good light path	
	Easy to clean up	
	Good for immobilization of algae	
	Large illumination surface area	

2.3.3 Harvesting and concentration

After microalgae culture has reached a stationary growing phase (i.e. slow growth rate and cells mobility) in suspension within the water medium, the biomass produced is recovered from the water before undergoing further downstream processing, such as extraction of fuel precursors and their conversion to biofuels. Harvesting requires different steps and approaches, depending on the features of the selected strains, e.g. size and density, as well as target concentration in the final slurry.

The harvesting stage usually contributes to one of the main costs associated to microalgal production applications [78].

Generally, the concentration of microalgae in ORPs or PBRs is likely to be less than 0.1% (i.e. mass concentration less than 1 g/l). After harvesting and concentration, microalgae need to be concentrated to a wet paste containing at least 10-25% of total suspended solids (TSS, in mass), depending on the selected downstream processing technology [11, 12, 62]. So far, many experimental studies have analysed various microalgae harvesting techniques, including chemical, biological and physical methods, or combinations. The main aim is to improve the biomass recovery efficiency while reducing the process costs [78].

A **screening pre-treatment** may be applied to pre-concentrate the microalgal culture from the initial concentration of 0.01-0.15% TSS, depending on the cultivation system, to a concentration of 5-6% TSS [78] before implementing the harvesting steps. In the screening step, the microalgae flow stream is processed through vibrating screens of various meshes, in continuous or batch mode [78].

Next, the harvesting phase generally includes two main processes, namely: i) **thickening**, where the microalgae suspension is transformed into a slurry of about 6-10% TSS; ii) **dewatering** to convert the processed slurry to an algal paste containing 10-25% TSS. Next, a drying stage can be implemented to increase the solids concentration level of the final biomass stream. This will depend on the selected downstream processing technology to convert the algal biomass to a specific biofuels product, e.g. biodiesel, bio-oil, see next section 3 for further details.

An overview of the main thickening options for microalgae processing is given below:

Chemical coagulation/flocculation: these methods involve the manipulation of the biomass suspension, by means of pH adjustment or addition of chemical coagulants or flocculants to the broth. This approach mainly promotes the agglomeration of the algal microscopic cells into large algae aggregates that will be settling afterwards by gravity sedimentation. Efficient interactions between the algal cells and coagulants/flocculants (e.g. chloride, sulphate, aluminium salts, calcium hydroxide solutions) depend on the characteristics of the algal cells, such as their surface properties and concentration in the processed streams. A detailed analysis of specific experimental set-up and recovery efficiency of different strains is reported in a recent study [78]. Overall, coagulation/flocculation followed by sedimentation is a technologically simple approach; though costs for chemicals may be a limiting factor. In addition, the treated biomass can be contaminated by the chemicals used. Also, the relationship between coagulant dose and cells concentration has not yet been fully clarified by experimental work.

Electricity based processes: the microalgae cells, which are negatively charged, can be separated from the water medium by applying an electrical field to the broth (avoiding the addition of chemicals). In these conditions, the algal cells may precipitate on the electrodes (electrophoresis) or accumulate on the bottom of the vessel (electro-flocculation). An overview of the types of electrodes, flocculation mechanisms and parameters affecting the efficiency of the electricity based methods has been reported in [78]. Based on the literature information, the electrical approaches are applicable to a wide range of microalgal strains. However, this method is more suitable for collecting microalgae of marine water origin compared to freshwater microalgae. This result is mainly related to the lower ionic strength of the marine water microalgae compared to that of the freshwater microalgae. This may enhance the efficiency of electrical harvesting method. On the other hand, it should be considered that these techniques require high energy consumption and equipment costs.

Autoflocculation/bioflocculation: the method implies the binding of the algal cells into algae aggregates without the use of chemical flocculants. The process may occur naturally when microalgal cultures are exposed to sunlight, under limited CO_2 supply and pH conditions between 8.6-10.5. Moreover, bioflocculation can imply the addition of bacteria and fungi or higher organisms than algae, such as shrimps, that may facilitate their harvesting and dewatering. The efficiency of this method mainly depends on the ability of microalgae to form aggregates in such environment. Detailed analysis of microalgae/fungi association is provided in the literature [78]. Despite their potential benefits, these methods are not yet implemented at large-scale, mainly because bioflocculation conditions may not be easily controlled and possible modifications of the quality of microalgae may occur [12, 79].

Following the thickening step, microalgal biomass needs to be separated from the growth medium by alternatively applying the following methods:

Gravity sedimentation: as mentioned before, the sedimentation step is generally designed after coagulation/flocculation processes, leading to the separation of microalgae from the water stream [78]. Sedimentation can be an efficient process for separating various microalgal strains, mainly depending on their density. Although, this process has may require considerable time to be completed [59].

Dissolved Air Flotation (DAF): as opposed to sedimentation, the DAF implies the separation of the algal biomass from the water medium bringing the algal cells to float to the surface. This effect is reached after gas bubbles are injected into the broth. Similarly to sedimentation, DAF is often implemented after coagulation/flocculation steps. Efficiency of this method is enhanced for hydrophobic cells that are small in sizes, as they can be easily attached to the air bubbles. Furthermore, the efficiency of DAF may be affected by the size and flux distribution of the air bubbles through the microalgal suspension.

The microalgal slurry from the thickening step generally undergoes a dewatering step to enhance the concentration of the harvested biomass that will be further processed in downstream steps. Dewatering can be performed by means of several alternative methods, including:

Filtration: this process can be used in combination with previous thickening and separation steps or even without them, depending on the characteristics, such as size and density, of the selected algal strain. In this process, the liquid media containing microalgae in suspension is basically forced through a membrane with appropriate pore size, under constant pressure drop. Despite its conceptual simplicity, filtration is an expensive process and presents many challenges. Among these is fouling, i.e. deposit of microalgae on the membrane, reducing the filtration rate. For this reason, membranes must be regularly cleaned to ensure appropriate biomass recovery rates. Different membrane configurations have been designed, namely microfiltration (MF) and ultrafiltration (UF) membranes that are suitable for fragile cells. Laboratory/pilot scale research has been recently carried out for vibrating and conventional cross-flow MF and UF membranes systems [80]. The performances of vibrating membranes seem promising, enhancing the overall biomass recovery. On the other hand, these types of membrane are costly and energy intensive, and need frequent replacements [78]. Moreover, MF and UF processes can be cost effective only in the case of small volume to be treated (below 2 m^3/d) [78]. Another important step of the filtration process is the recovery of algal biomass from the filter. Washing the filter might cause the re-dilution of the biomass product. Hence, innovative and cost-effective designs need to be further researched, considering only limited or no washing step [12].

Centrifugation: this technology, which is widely used in industrial applications for solid to liquid separation, has been generally considered for collecting microalgae from the water medium [21, 59]. As for the filtration method, a centrifugation step can be used in combination with the thickening and separation phases or even without them. If the process is applied after thickening and separation of the algal cells from the water medium, its energy consumption is lowered thanks to the reduced volumes to be processed. Furthermore, a long retention time is needed to enable high biomass separation efficiency, due to the small size of the algal cells. Overall, centrifugation requires large initial capital investments, operating costs and energy inputs. The current development of this technology and costs seem prohibitive for its implementation in large-scale microalgae production systems [78].

Next, biomass drying may possibly be implemented (optional) to enhance the efficiency of the downstream processes, such as the extraction of lipids from dried algal biomass and conversion to biodiesel (see chapters 3 and 4 for further details). The heat required for drying may be obtained from different sources, namely: natural gas fed drum dryers and other oven dryers. Depending on the climate humidity and temperature, solar or wind drying would be beneficial in reducing the energy input and costs of the entire microalgae-to-biofuels value chain. On the other hand, the major limitations to the large-scale development of these systems generally include the requirement of long times and large surfaces, as well as risk of material loss [11].

Figure 2.11 schematically shows the previously described harvesting methods, namely thickening, dewatering and possibly drying, to separate and recover microalgae from their growth medium, enabling their conversion to biofuels and/or bioproducts.

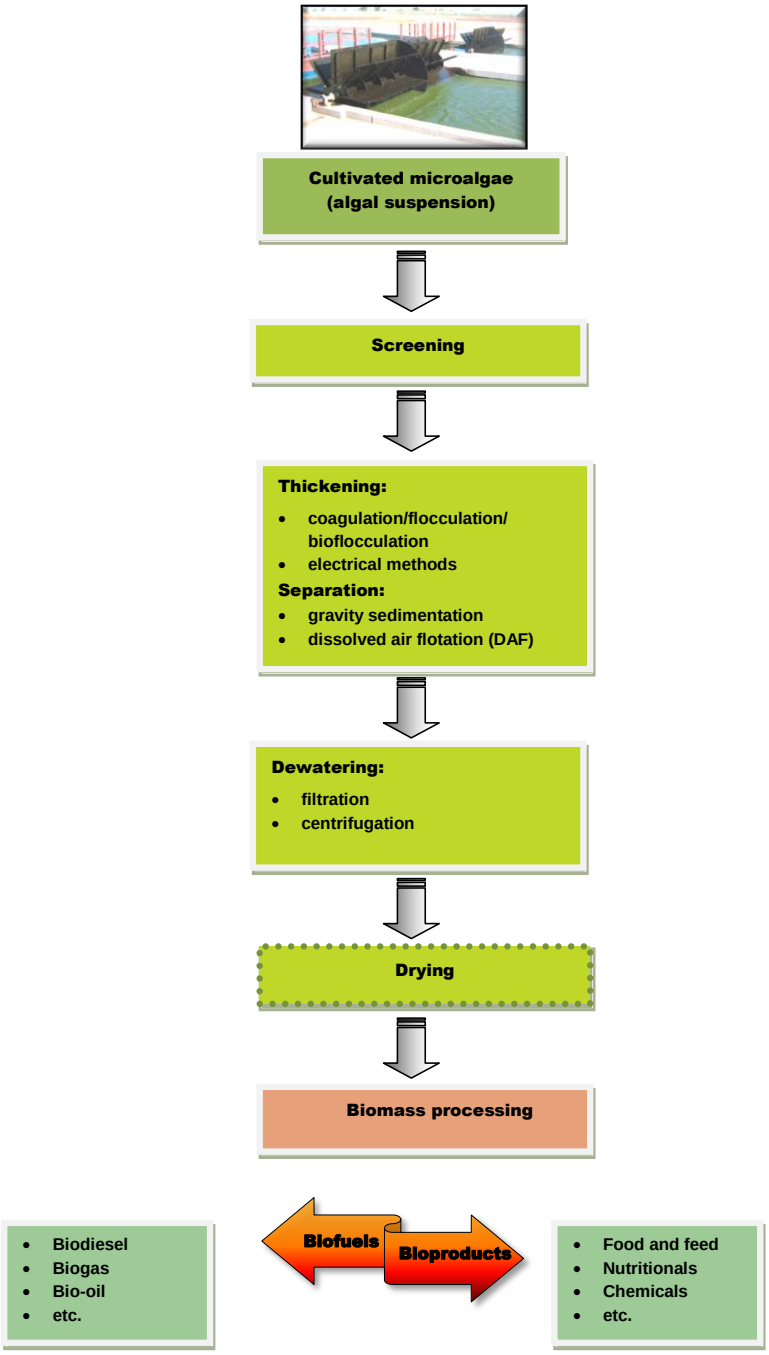

Figure 2.11. Process flows diagram of harvesting methods for recovery and dewatering of microalgal biomass. Drying is optional, depending on the selected downstream process for conversion of microalgae to biofuels and/or bioproducts.

Furthermore, an overview of the microalgal biomass recovery efficiencies, energy consumption and costs (only limited analysis of costs are available) associated to different harvesting and dewatering methods of various microalgae strains is given in Table 2.3.

High recovery efficiency of 80-99% can be obtained by means of different thickening methods followed by dewatering, for various strains. However, high variation of the

results, i.e. between 32-92% can be observed when bioflocculation+sedimentation are applied to specific strain (*Chlorella vulgaris*). The efficiency of this specific method significantly depends on the conditions of the growth medium (pH, temperature, salinity) and interactions between the harvested microalgae and the fungi/microorganism utilized as flocculent agent in the system (bioflocculation).

The results in Table 2.3 also indicate that, for different strains, centrifugation requires higher energy input compared to other harvesting methods. A relatively high energy input, 72 MJ/m^3, is required to obtain 96% of algal biomass recovery. Detailed description of the technology set-up and operating parameters of the alternative harvesting techniques can be found in a recent work [78].

After the harvesting and dewatering stages, microalgal stream usually containing 10-25% of solids is obtained. Therefore, 100-250 tonnes of water are removed per each tonne of produced microalgae. In view of this, it is important to select suitable harvesting methods that would enable the water recycling to the algal cultivation system. In addition, the harvesting techniques must preserve the biomass quality for the enhancement of its conversion to selected biofuels/bioproducts in the downstream processing.

For the time being, there is no consensus on the optimum harvesting method for microalgae, in terms of applicability, environmental impacts and cost efficiency [79]. On the other hand, lowering the harvesting costs, while improving the biomass recovery are regarded as key factors for enhancing the sustainability of the whole microalgae production chain [12, 32].

In existing commercial applications, such as food and feed industries, the most common microalgal harvesting methods include flocculation, sedimentation, filtration and centrifugation [59]. Future efforts are needed to adapt the most common harvesting/separation technologies used in microalgal commercial sectors to the biofuels sector. The development of harvesting applications for potential large-scale microalgal cultivation technologies present critical issues [78]. For the time being, developing and implementing viable and cost-efficient harvesting technologies at large-scale still remain a major challenge for the development of microalgal production chains [23].

Table 2.3. Recovery efficiency of microalgal biomass (in %), energy consumption (in MJ/m^3) and costs (USD/ton) of harvesting techniques for various microalgal strains [78].

Microalgal species	Harvesting	Efficiency	Energy consumption	Costs
		%	MJ/m^3	USD/ton
Chlorella vulgaris	• Coagulation/flocculation+sedimentation	92-99	n.a.	n.a.
	• Autoflocculation+Sedimentation	98	n.a.	18
	• Bioflocculation+Sedimentation	34-99	n.a.	n.a.
	• Filtration	98	0.972	n.a.
Chlorella minutissima	Coagulation/flocculation+sedimentation	80	n.a.	n.a.
Chlorella sp.	Flotation	90	n.a.	n.a.
Chlorella sorokiniana	Coagulation/flocculation+sedimentation	99	n.a.	200
Dunaliella salina	• Flocculation+flotation	98.2	n.a.	n.a.
	• Electrolytic Flocculation	98.9	0.828	n.a.
Tetraselmins sp.	• Electro-Flocculation	87	0.559	n.a.
	• Electro-Flocculation+sedimentation	91	0.328	n.a.
Nannochloropsis oc.	Bioflocculation+Sedimentation	88	n.a.	n.a.
Nannochloropsis sp.	Centrifugation	96	72	n.a.
		17	2.88	
Scenedesmus sp. and *Coelastrum rob.*	Centrifugation	2-15	2.6-3.6	
Phaeodactylum tr.	Coagulation/flocculation+sedimentation	67-91.8	1.19	0.429-1.429[(a)] 0.976-2.073[(b)] 2-100[(c)]

n.a.: not available information; [(a)]: USD/kg coagulant (polyaluminium chloride-PAC); [(b)]: USD/kg coagulant (Al_2SO_4); [(c)]: USD/kg coagulant (Chitosan).

2.3.4 Current applications and future perspectives

Several studies reported that a number of microalgae can be efficiently grown in ORPs using wastewater (WW) effluent (which is normally processed in a WWT plant) as a source of low-costs water and nutrients [14, 81, 82]. Furthermore, microalgae showed a high accumulation capacity for different metals (e.g. selenium, chromium, lead) and organic toxic compounds (hydrocarbons) that are present in WW effluents. A detailed description of the pollutants remediation capacity measured for selected microalgal strains can be found in recent studies [11, 65].

In view of the above, researchers generally consider the combined use of microalgal production and WWT system as the most attractive option for the implementation of algal biofuels applications. However, production of biofuels from microalgae grown in WW effluent can make only a minor contribution to the liquid fuel supply. In these conditions, the potential of the algal fuel is limited by the availability of the WW effluents and the yield of biomass grown in WW effluent, i.e. sub-optimal conditions for algal growth and lipids accumulation. According to [81], algal oil from microalgae grown on WW produced by a large US city of 10 million may equal up to 3% of the fuel requirements of the same city for transportation.

Furthermore, microalgae showed the potential of capturing CO_2 from flue gases produced by power plants/cement manufacture industry [11, 65]. Flue gas containing 15-20% by volume of CO_2, at the temperature between 20-35 °C, can be used for optimal growth of algae. However, no inhibition to growth was found at CO_2 concentrations level above 20% in the flue gas [83]. The temperature of flue gases from conventional power plants is around 65-95 °C, therefore flue gas cooling step is required. Carbon capture efficiencies of microalgae might vary between 45-70% in closed cultivation systems and between 25-50% in open systems [65, 83].

The combined production of algal biomass with CO_2 reusing option is considered a potential cost-effective application by many researchers and companies. However, selection of suitable strains for CO_2 capture is of extreme importance for the efficiency of the utilization process. According to information in the literature, only a limited number of microalgal strains are tolerant to high levels of SO_x and NO_x that are contained in flue gases [11]. Furthermore, high water temperature tolerance should be achieved by the selected microalgae to minimise the costs of cooling exhaust flue gases.

The potential benefits of using CO_2 from combustion processes for microalgae biomass production may reduce the costs of the algae production. However, the quantity of CO_2 that is absorbed by the algae during growth is emitted during the combustion of the algal biofuels. Therefore, unless the carbon in the algal biomass is geologically stored, the emissions from the combustion process are actually not reduced, but rather the algal biofuels can be considered fully carbon neutral.

3 Biofuels from algae

In the context of developing renewable biofuels technologies, macroalgae and microalgae feedstocks are considered attractive feedstocks with many advantages over terrestrial crops, such as corn, sugarcane and soybean, as well as lignocellulosic feedstocks, such as agricultural residues and wood waste. A variety of algal species can be grown in salty/brackish water or even in WW effluent, contributing to the improvement of the water quality by removing nutrients and metals, as mentioned in previous section 2.3.4. Moreover, algal cultivation may take place on non-arable land. These aspects represent a clear theoretical advantage for algae-based biofuels over first, or second, generation biofuels. Furthermore, algae biomass lacks the recalcitrant lignocellulosic components, thus, it can be converted to fuels relatively easily if compared to lignocellulosic feedstocks.

In view of the above, it is of interest to investigate the potential use of algae as a biomass feedstock for production of bioenergy and/or biofuels for transport, such as biodiesel, biomethane and bioethanol, among others. The production of biofuels is particularly appealing because of the almost total dependence of transportation sector from liquid fossil fuels.

The selection of the algae-based conversion pathways for production of usable energy and transportation fuels is dependent on different parameters, such as: chemical characteristics of the considered algal species, site-specific local conditions (in terms of resource supply and biomass yield), as well as the desired end-products.

Generally, macroalgae are considered for the production of biogas and bioethanol via fermentative process rather than biodiesel. The reason is that these species generally do not contain high amounts of lipids/oils from which the biodiesel is mainly derived via biochemical or thermochemical processes. According to literature, the biodiesel yields of macroalgae are much lower than those of microalgae [53].

During the last two decades, a lot of research has been carried out on microalgae-based biofuels. Considering their enormous variety and versatility in composition, research focused on the use of microalgae for production of biodiesel, biogas and bioethanol. Microalgae have high potential for biodiesel and biogas production, due to their lipids accumulation capacity. Microalgae have been also considered as a feedstock for ethanol production through fermentation processes, although this pathway is less investigated than biodiesel and biogas [84, 85]. In addition, other potential biofuels from microalgae include: bio-oil (or "biocrude") and hydrocarbon bio-liquids (to be converted in renewable diesel, gasoline and jet fuel) that are obtained via thermochemical processes and bio-hydrogen that can be produced under dark fermentation conditions. [21, 32, 49].

The production of each liquid and/or gaseous biofuel requires proper characterisation of the algal feedstock and conversion processes. Moreover, it should be considered that the production of bioenergy and/or biofuels in combination with biomaterials (e.g. chemicals, nutraceuticals, fertilizers and animal feed) in integrated biorefineries is a key requirement for macro- and microalgal production. To this regard, t well recognized that focusing on a single product/application would unlikely make the use of algae sustainable from an economical and environmental point of view [20, 32].

In this chapter we aim to synthetize the literature on possible biofuels pathways for both macroalgae and microalgae species, in terms of technological development and limitations based on recent research efforts made in the field. These include the conversion of:

- macroalgae to: biomethane, bioethanol and biobutanol;
- microalgae to: biodiesel, biomethane, bioethanol, bio-oil (or bio-crude) and bio-hydrogen.

3.1 Macroalgae based biofuels options

3.1.1 Biomethane

Among the possible biofuels options, AD for the production of biogas, i.e. primarily a mixture of methane and carbon dioxide, from macroalgae is considered one of the most viable technologies. Considering the high moisture (85-90% wt.) and fermentable carbohydrates content of macroalgae (23-79.4% by dry wt., as shown in section 2.2.1), AD can be an efficient technology for their conversion to a biofuel. In addition, macroalgae contain little cellulose and normally no lignin (i.e. recalcitrant fractions), depending on the species [86]. Therefore, macroalgae generally may undergo a more complete hydrolysis compared to terrestrial crops [53].

The produced biogas can be used in a combined heat and power (CHP) system, for production of heat and electricity, or upgraded to biomethane and then compressed or liquefied for use as transportation fuel.

AD involves different decomposition phases, in which specific bacterial communities contribute to the degradation of the substrate. The technical viability of biogas production from seaweeds via AD has been demonstrated in several experimental tests that were mainly conducted at the laboratory/pilot-scale, see e.g. [37, 87-92]. It was generally observed that various species exhibit high biomethane potential, conversion efficiency and stability of the digestion process [38, 93, 94]. The potential biomethane yield of macroalgae can range between 0.08-0.40 m^3 of CH_4/kg VS, mainly depending on the biochemical composition of the species (see Table 3.1) [53, 94, 95].

Nevertheless, several technical and economic challenges for macroalgae-based biogas production are identified [38, 53]. Among these, the fluctuation of the macroalgae supply over the year, depending on the time of harvest and location of the culture, is a critical factor to be considered for the techno-economic viability of macroalgae-based AD for biogas production.

Also the seasonal variations in the chemical composition of seaweeds will affect their conversion to biogas. This effect was observed for brown macroalgae (such as Laminaria sp. and Saccharina latissima from the Norwegian costs), during a series of bench-scale culturing experiments [91, 94]. It was found that the chemical composition may greatly vary depending on the season. In general, the carbohydrates content of macroalgae is high during summer and autumn. This can be explained by the fact that, in these periods, carbon accumulation via photosynthesis exceeds carbon utilization [96]. Therefore, the authors concluded that the harvesting time of the considered species may be regarded as a key parameter for optimizing the biomethane yield [91]. To overcome this issue, ensiling is a promising method for preservation and storage of high-methane potential seaweeds for continuous biogas production throughout the year [97].

Furthermore, seaweeds may contain high amounts of proteins resulting in low (C/N) ratios (below 20). To obtain high yield of biomethane, optimal C/N ratios of about 20-30 are required. In fact, when C/N is lower than 20, the microbial growth can be inhibited by the high levels of ammonia formation during AD. The results of a previous study showed that some species (e.g. *Laminaria sp.*) ranged from low C/N in spring to high C/N in autumn [38]. These results confirm that the seasonal variations in seaweed composition may lead to high variability of the biomethane potential of these species.

The chemical composition of the algal substrate is also a limiting factor of AD for biogas production, affecting the structure of the bacterial community that is active at each step of the process. The equilibrium of the different process steps can be altered when some toxic compounds are formed during the process, leading to the disruption of the involved microbial activities. The degradation of macroalgae and their subsequent conversion to biogas via AD also depend on other variables, such as the: selection of inoculum, temperature, operational parameters and design of the digester. Cow manure inoculum was found suitable for macroalgae (*Macrocystis pyrifera*, *Durvillea Antarctica*) [93].

The results of experiments at various temperature ranges (such as psycrophilic, mesophilic and thermophilic intervals) of macroalgal AD showed that mesophilic conditions (up to 35 °C) improve the biomethane production of green seaweeds [98].

Operational parameters of the AD process, such as the hydraulic retention time (HRT, in days) and organic loading rate (OLR, in g VS/l) also affect the attainable biomethane production of seaweeds. These parameters need to be chosen depending on the type and composition of the algal substrate. The HRT would depend on the existing recalcitrant organic matters and degradability of the biomass. A wide range of HRT values between 8 and 64 days may be required for effective processing of (macro and micro) algae [99, 100]. The results of a recent study indicate that HRT of 20 days would be sufficient to obtain nearly maximum digestibility of different seaweeds substrates [94].

The OLR values can range between 0.74 and 11.2 g of VS/l. Results indicate that the biomethane yield increases when OLR decreases and HRT increases. However, high HRT values may imply an increase in the costs of the AD processes. To this regard, it was observed that by decreasing the reactor volume and HRT, while increasing the biomass loading rate might represent a valuable option for the economics of AD processing [83].

The produced biogas manly consists of methane, carbon dioxide and small proportions of hydrogen sulphide gas, depending on the composition of the selected species and operational AD conditions. The results of studies on seaweeds indicate methane proportions ranging between 49-78% of the biogas [87, 101, 102]. Estimates of the biomethane yields (m^3 of CH_4/kg of VS) of different macroalgal species that were processed under different AD conditions are reported in the Table 3.1. The biomethane yields vary depending on different parameters, including HRT and OLR conditions [88-92, 101, 103].

Table 3.1. Methane yields (in m^3 of CH_4/kg VS) of different macroalgal species [88-92, 101, 103]. Information on the AD reactor configurations and process designs are presented, including the: type of reactor (continuous stirred tank reactor-CSTR; semi continuous and batch), HRT and OLR.

Macroalgae	Reactor type	Volume (l)	HRT (days)	Temperature (°C)	OLR (gVS/l/day)	Methane yield (m^3CH_4/kg VS)	References
Ulva sp.	CSTR	50	26	37	1.9	0.15	[90]
Ulva sp.	CSTR	1	25	37	1.6-1.85	0.08-0.11	[101]
Ulva sp.	CSTR	6	30	37	1.04-1.25	0.19-0.29	[101]
Ulva sp.	CSTR	1	20	30	1.47	0.12-0.20	[88]
Ulva sp.	CSTR	5000	12-20	35	1.85-2.66	0.15-0.38	[89]
Ascophyllum n.	semi continous	10	24	35	1.75	0.11	[91]
Laminaria h.	semi-continous	10	24	35	1.65	0.23-0.28	[91]
Laminaria sacch.	semi-continous	n.a.	40	na	n.a.	0.22-0.27	[92]
Graciliaria sp.	batch	n.a.	n.a.	35	n.a.	0.28-0.40	[103]
Sargassum fl.	batch	n.a.	n.a.	35	n.a.	0.18	[103]
Sargassum pt.	batch	n.a.	n.a.	35	n.a.	0.15	[103]

n.a.: not available

The methane yields of macroalgae can be improved by applying mechanical pre-treatments prior to AD [79, 95]. Combination of washing and grinding can be suitable to enhance the biodegradability of the algal substrate via hydrolysis. Methane yields of

various washed and macerated species can be increased from 17 to 68% compared to the values resulting from the untreated samples [83]. The effects of pre-treatments mainly depend on the composition of the selected species. In addition, it is necessary to consider to which extent such pre-treatments may affect the overall economic and environmental viability of the process. These aspects must be further developed in future studies. Also, there might be potential for improving the biogas yields through co-digestion of some species of macroalgae with a substrate that is richer in nitrogen (such as manure) and manipulation of the microbial composition of the inoculums [95].

3.1.2 Bioethanol and biobutanol

Macroalgae can be suitable substrates for bioethanol production via hydrolysis followed by fermentation, due to their significant amount of carbohydrates, mainly glucose, galactose and mannitol, as well as little quantity of lignin.

The main options for hydrolysing seaweeds include the treatment with: i) sulphuric acid (H_2SO_4) at high temperature and ii) specific enzymes, such as cellulase, xylanase, and glucosidase, that facilitate the release of sugars during the process [46, 53].

The average percentage of sugars that can be released from total carbohydrates contained in seaweeds may significantly vary depending on the species and the hydrolytic treatment used. The released sugars may undergo microbial fermentation to produce bioethanol and/or biobutanol [53]. Results of experimental tests on microbial fermentation of brown seaweeds indicated an ethanol yield of 7.0-9.8 g/l from 50 g/l of sugar within 40 h, under acidic conditions [104]. As for the butanol processes from green macroalgae (*Ulva*), the average yield corresponded to 4 g/l out of 15.2 g of sugars/l under acidic hydrolysis [104].

Similarly to biogas production, pre-treatments of macroalgae may improve the overall efficiency of the fermentation process for ethanol production. Mechanical and/or acid pre-treatments using hydrolytic enzymes may improve the hydrolysis of the macroalgal sugars, while enhancing the ethanol yield of the process [53]. The fermentation efficiency of macroalgal sugars for ethanol production may also be improved by using metabolic engineering strategies. Research efforts in this area are scarce, though promising results have been obtained so far [46].

Appropriate utilization strategies for by-products of macroalgal fermentation, such as glycerol and organic acids (e.g. acetate and succinate), need to be considered to enhance the economic value of the seaweed-based fuel production chain. Produced organic acids can be employed in the food industry, while glycerol may be applied in the food manufacturing or in the pharmaceuticals sector. However, there is currently an over-supply of glycerol from first generation biofuels production. Realistic assumptions on the market value of glycerol are, hence, required when evaluating the economics of the overall process.

In Europe, research and demonstration activities on seaweed-based biofuels strategies have been carried out in the framework of the BioMara project (http://www.biomara.org/meet-the-team-1/cultivating-seaweeds), an EU-funded project developed from 2009 to 2012, under research and industry partnerships in the UK and Ireland. The project aimed at investigating solutions for production and potential commercialization of seaweed-based ethanol and derived co-products in different industrial sectors. The analysis concluded that macroalgae cultivation have no major biological obstacles along Scotland's coasts [45]. However, an improved understanding of the environmental impacts of large-scale seaweeds farming are needed through further pilot projects. On the other hand, considerable technological advancement is required to mechanise the planting and harvesting of potential large-scale macroalgal cultures. So far, research conducted on macroalgae-based farming and processing is relatively scarce if compared to that on microalgae feedstock. Hence, further research in this field is needed.

The current poor costs-effectiveness of the macroalgae-based technology limits its viability in the biofuels industry. The estimated cost of macroalgae-based ethanol is about $ 0.50/kg (dry wt.), which is higher than that of corn ethanol, corresponding to about $ 0.16/kg (dry wt.) [53]. Hence, the costs of macroalgal feedstocks cultivation and processing for biofuels production need to be reduced by at least 75% of the present level to become an attractive option.

3.1.3 Other biofuels types

Besides biogas, bioethanol and biobutanol, macroalgae can be converted into biodiesel, bio-oil and bio-hydrogen via appropriate biochemical and thermochemical conversion technologies [39, 53, 105]. However, as discussed before, biogas/bioethanol/biobutanol are considered the most suitable biofuels options that can be produced from macroalgae. This is related to the high content of moisture and carbohydrates in seaweeds that can be converted to biofuels by means of wet conversion methods, including AD and fermentation.

Nevertheless, some attempts have been made to produce biodiesel from macroalgae, as reported by [53]. In experimental studies a two-steps esterification method, using acid catalyst, for conversion of free fatty acids into biodiesel was developed. However, biodiesel yields that can be obtained from macroalgae are much lower than those of microalgae [53]. Therefore, compared to microalgae, less attention has been paid to the use of macroalgae for biodiesel production. To date, there is no detailed analysis indicating the potential technical and economic viability of biodiesel pathways from macroalgae.

The use of thermochemical conversion process for the production of bio-oil from macroalgae was also investigated. Also in this case, less research has been performed on such conversion routes for macroalgae compared to microalgae (see next section 3.2.5 for further details on thermochemical conversion of microalgae). A few experimental studies investigated the suitability of brown, red and green macroalgae to be converted into bio-oil via pyrolysis of the whole biomass. The process is likely to be tolerant to the high ash content of macroalgae. On the other hand, a major limitation to the viability of pyrolysis is the high moisture content of macroalgae, which is about 85-90% as reported in before in section 3.1.1, requiring considerable energy for drying the biomass before the implementation of the process [39, 53]. The conversion of macroalgae through pyrolysis can also be hampered by their high amounts of mineral components, e.g. Ca, K, Na and Mg, that can lead to high char formation, while lowering the yield of bio-oil. The application of pre-treatment steps may be considered to remove such components [39]. The bio-oil yields may significantly vary depending on the macroalgae composition and operating conditions of the pyrolytic process, such as temperature and heating rate. As a result, large variations in the bio-oil yields between 11-49% were observed, depending on the selected species. Pyrolysis of macroalgae at 500 °C has been demonstrated to achieve the maximum yield of bio-oil [53].

Hydrothermal liquefaction (HTL) for bio-oil production from macroalgae has also been investigated in studies mainly based on experimental batch tests [38]. HTL is carried out using supercritical water (at 200-400 °C) as a reaction medium in the presence of a catalyst, see detailed information given in next section 3.2.5. Differently from pyrolysis, HTL can be suitable for wet biomass, requiring no drying of the treated feedstock. Experimental studies on macroalgae obtained a bio-oil yield varying between 8.7–29.4%, mainly depending on the biochemical composition of the selected macroalgae [38, 53, 106]. The maximum conversion to biocrude was at 360 °C for Alaria esculenta, as reported in [106].

Overall, lower bio-oil yields can be obtained for macroalgae compared to microalgae. This is generally attributed to higher ash and lower lipids content of macralgae compared to microalgae. Furthermore, the high proportions of carbohydrates (and low content of lipids) in macroalgae can enhance the formation of char, while lowering the liquid bio-oil yields. The produced bio-oils are mainly composed of fatty acids and esters, with

generally low heating values due to the high contents of oxygen, sulphur and nitrogen [53]. Thermochemical technologies for conversion of macroalgae to biofuels are currently in the phase of basic research. Therefore, at present, there is lack of proper assessment of potential bio-oil production from macroalgae, in terms of environmental impacts and cost-effectiveness.

Macroalgae can also be a suitable feedstock for biohydrogen production [105]. The process can occur via dark fermentation by means of a pure or mixed culture of hydrogen-producing bacteria. A recent study reports the biohydrogen potentials of (red, brown and green) species to vary between 10.3-67 ml of H_2/g of TS depending on the considered species [105]. Biohydrogen production from macroalgae is indicated to be limited by the hydrolysis of carbohydrates within algae. Effective pretreatment steps can enhance the conversion of complex carbohydrates into simple sugars. However, the use of cost effective biomass pretreatments is considered one of the most challenging step in the overall biomass conversion technologies [39]. The integration of dark fermentation for hydrogen production into a biorefinery, i.e. by using different conversion processes of macroalgae substrates for co-production of biofuels and high-value compounds (e.g. products for human consumption and chemicals), is foreseen as an approach that can improve the process viability [39, 105]. Production of bio-hydrogen from seaweed is still at an early stage of development. Indeed, the available information is limited to the conversion yields that can be obtained at lab scale. For that reason, proper energetic, environmental and economic assessments are still lacking in the current studies.

3.2 Microalgae based biofuels options

3.2.1 Biodiesel

Biodiesel from microalgae has been widely explored in the last decades, and is considered to be among the most viable biofuel production pathways [10, 12, 21, 32]. This is because various microalgal species (e.g. *Scenedesmus d.*, *Nannochloropsis sp.*, *Chlorella v.*) are able to produce high amounts of lipids, hydrocarbons and other complex oils that can be processed into biodiesel (see data in previous Table 2.1) [10, 32]. Compared to terrestrial oil crops being exploited as biodiesel sources, microalgae are characterized by a higher oil yield potential, namely, the mass of oil that can be produced per unit area, as indicated in Table 3.2. On the other hand, differently from terrestrial crops, the extraction of oil from microalgae is difficult due to the presence of thick cell walls that can obstruct its release. Many studies have focused on the lipids extraction method that is carried out on harvested algal biomass (see previous section 2.7 for details on the harvesting process). According to literature, two lipids extraction methods can be performed, including the: i) chemical solvent extraction for dry biomass (50-98% dry wt.) and ii) supercritical fluid extraction for wet biomass (12-30% dry wt.) [70, 107].

The chemical extraction, which mainly uses n-hexane, chloroform and methanol or a mix of them as solvents, is the most common method, as it shows high efficiency in solubilising the microalgal lipids. However, the extraction efficiency may vary depending on the algal strains considered and the solvent used [70]. In addition, significant energy inputs are required for the drying of microalgal biomass which is necessary for the effective application of this technology [24].

Recently, researchers have focused on extraction methods employing supercritical fluid because they can be performed on wet microalgal biomass. Supercritical fluids, which are currently under investigation, include ethylene, CO_2, ethane, methanol, ethanol, benzene, toluene and water [70]. The main advantage of this method includes the reduced energy required for extraction when compared to the conventional solvent extraction. This is due to the removal of the drying step after harvesting. However, this method yields lower lipids compared to the chemical extraction method applied to dry biomass. For instance, experimental tests found that maximum extraction efficiencies of 70% can be achieved at about 220°C using subcritical water [108]. Further, these

technologies are associated with high of operation and safety related issues [70]. For the time being, they still require considerable research efforts before they can be implemented at industrial scale.

Following, the extracted microalgal lipids can be converted to biodiesel by conventional transesterification (alcoholysis) process. Various catalysts (base and acid) can be used to accelerate the process. Alternatively, in-situ transesterification can be performed, consisting in lipids extraction by chemical method, followed by transesterification occurring in a single step. The main advantage of this technique includes the possibility to optimize the use of the solvent, because it would be employed as a reactant in both the extraction and the transesterification steps [70]. Research performed at laboratory-scale on dried microalgae samples (e.g. *Chlorella v.*) has shown 90% efficiency in converting the extracted lipids to biodiesel by means of in-situ transesterification [109].

Microalgae-based biodiesel has similar physical and chemical properties compared to petroleum diesel and first generation biodiesel, complying with the criteria set in the International Biodiesel Standard for Vehicles (EN14214) [11]. Biodiesel from microalgae is considered non-toxic, and produces low levels of particulates, carbon monoxide, hydrocarbons and SO_x during its combustion (in internal combustion engines. Considering the overall features of the microalgae-based biodiesel, its exploitation potential in the aviation industry also seems promising [11, 32].

As already mentioned in other sections of the report, microalgal biodiesel is not commercial yet. The lack of large scale proven technology and high costs of the microalgal lipids production and conversion to biodiesel are the main obstacles to its potential commercialization [68].

Table 3.2. Estimated oil yield potential, namely, the mass of oil that can be produced per unit area, from terrestrial crops and microalgae [10].

Crop	Oil yield (l/ha)
Corn	172
Soybean	446
Canola	1190
Jatropha	1892
Coconut	2689
Oil palm	5950
Microalgae (a)	58,700
Microalgae (b)	136,900

a) Considering 30% of oil (by wt.) in microalgal biomass;
b) Considering 70% of oil (by wt.) in microalgal biomass.

3.2.2 Biomethane

In the last decades, many experimental studies, which were mainly performed at laboratory scale, tested the potential biogas production from various microalgae species via AD [99, 100, 110-112]. Microalgae are considered valuable substrates for biogas production due to their high content of lipids, carbohydrates and proteins and low amount of lignin, i.e. recalcitrant compounds, up to 5% of dry wt. [110, 113]. In addition, also the residual algal paste after lipids extraction (also defined as lipids-extracted algal, LEA, biomass) can be treated by AD. LEA biomass contains mainly carbohydrates and proteins residuals from the original feedstock [111, 114].

The results indicate that biogas yields of whole microalgae vary between 0.180 and 0.587 m^3/kg VS, mainly depending on the degradability of the species and technology-related conditions (HRT, OLR, digester design) [63, 110]. Biogas yield from LEA biomass

is about 0.300 m/kg VS under optimal laboratory conditions and HRT of 15 days [114]. Methane content in LEA biogas is about 55-70% [63, 110, 113]. This can be compared with methane yields of about 0.2 m^3 of CH_4/kg VS for slurry manure and about 0.35 m^3 of CH_4/kg VS for maize silage [115].

Produced digestate from AD, of either the whole microalgae or LEA biomass, may be separated into the liquid and solid fractions. The liquid fraction, which mainly contains soluble nutrients components, may be recycled to the microalgae cultivation system where it would displace synthetic nutrient inputs that are required for the algal growth. The solid fraction of the digestate may be used as fertilizer.

On the other hand, various microalgal species may contain a high proportion of proteins resulting in low C/N ratios. In these conditions, the AD of microalgal substrates would be difficult due to the excess of ammonia produced in the digester [37]. The performance of the AD may be improved by the co-digestion of microalgae with carbon-rich waste, such as waste paper and waste activated sludge [110]. A significant increase in methane production was achieved with the addition of waste paper to microalgal biomass. The methane production of a mix 50% waste paper/50 % algal biomass has been found to be twice the one of AD of pure algal biomass [11]. An improved biogas yield and biodegradability rate was reported also for microalgal species when co-digested with waste activated sludge [63, 100, 113].

The process step showing the highest energy demand is heating of the digester [63]. Moreover, depending on the selected species, significant hydraulic retention time (HRT) of 20-30 days may be required to achieve maximum degradability. As mentioned in section 3.2, high HRT values imply increased cost of the overall AD installations [99].

In addition, the production of 1 MJ of biomethane from microalgae can require a larger area of land compared to that required for to the production of 1 MJ of microalgae-based biodiesel [63]. Therefore, the conversion of residual LEA biomass to biogas in combination with biodiesel production is foreseen as a more promising option compared to the AD of the whole microalgae.

3.2.3 Bioethanol

Similarly to macroalgae, microalgae can be suitable feedstock for bioethanol production [32, 63, 84]. The production of bioethanol from LEA biomass in combination with biodiesel generation can also be a viable option. However, so far, bioethanol production from microalgae has received less attention compared to biodiesel production.

Microalgal biomass can contain significant amount of carbohydrates (about 40-50% dry wt.) with no structural biopolymers, such as lignin and hemicelluloses [21, 32, 59]. Under specific conditions, microalgal carbohydrates can be degraded via hydrolysis and then fermented to bioethanol with yeast [22]. In the case of microalgae-based fermentation, it is possible to avoid chemical and enzymatic pre-treatments, which are energy intensive processes necessary for ligno-cellulosic feedstocks, to release the sugars contained in the algal cells. However, mechanical pre-treatments are still needed to break down the algal cells, e.g. disruption by high pressure homogenizer or collision plate [63].

Microalgae-based bioethanol production yields range between 0.240 and 0.888 g of ethanol/g of substrate, at the temperature of 25-30 °C [63, 84, 110]. The maximum conversion efficiency of microalgal biomass to bioethanol was found to be 65%, under optimum laboratory conditions [63]. The potential of microalgae as a feedstock for bioethanol production can vary significantly depending on the biomass species and pre-treatment steps (mechanical and/or chemical) used to hydrolyse the algal carbohydrates.

Bioethanol production is still in the preliminary research phase. Genetic modification of selected microalgal strains in combination with specialized bioreactors for ethanol production is being researched by Algenol Biofuels Company in Mexico. This approach

might provide valuable solutions for improving the effectiveness of the overall process [63].

3.2.4 Biohydrogen

Microalgae also received growing interest as a feedstock for hydrogen production. Production of hydrogen from different microalgal strains can occur via dark-fermentative process using a pure or mixed culture of hydrogen-producing bacteria [105]. Alternatively, hydrogen from microalgae can be produced via light-driven process, i.e. photofermentation, under anoxic conditions [32, 63]. These processes can either employ water or specific bacteria related to the fermentative conversion of biomass to hydrogen [32].

Carbohydrates and nitrogen-rich components, such as proteins, contained in algae are essential for the growth of hydrogen-producing bacteria [87]. On the other hand, excessive proteins content in algal biomass can lead to high release of ammonia that inhibit the activity of hydrogen-producing bacteria or specific enzymes related to fermentative hydrogen production [105]. Thus, chemical composition is a limiting factor for biohydrogen production from microalgae.

Recent studies indicate that, under dark fermentation, the potential degradability of various microalgae species can range between 2-28% [63, 83, 105]. Hydrogen yields resulting from experimental tests can vary from 13 to 48 ml of H2/g of algal substrates, depending on the composition of the selected species and operating conditions (such as pH, temperature, substrate/inoculums ratio) [99]. Biohydrogen yields, which were obtained during experimental tests, correspond to only 25-30% of the theoretical biohydrogen potentials calculated considering the degradability of all carbohydrates contained in the biomass [105].

Generally, the hydrolysis of carbohydrates and/or proteins, as well as the biohydrogen production can be enhanced by physical/thermal/chemical pre-treatments of algae. Thermo-alkaline (at 100-121 °C with 20% of NaOH) pre-treatments enhanced the solubilisation of both proteins and carbohydrates by 10-30% [105]. However, pre-treatment steps can be high-energy demanding and expensive depending on the specific biomass features and desired product.

Overall, the dark fermentation method for biohydrogen production leads to negative net energy balances, defined as the difference between the energy produced as biohydrogen and the energy consumed (i.e. heat and electricity) to produce it [105]. Thus, to make the overall process economically feasible, dark fermentation of algae must be integrated in a biorefinery approach, where the outputs/co-products are valorised into bioenergy and/or value-added biomolecules.

The combination of dark fermentation, photofermentation and AD in a three stages process can enhance the potential biohydrogen production from microalgae [99, 100]. Using such integrated approach, hydrogen yield of 198.3 ml H2/g VS and methane yield of 186.2 ml CH4/g VS were observed (for Nannochloropsis Oceanica). The production of such gaseous fuel products corresponds to an overall energy output of 2457 kWh/tonne VS [116]. The energy produced from the three-stages method was 1.7 and 1.3 times higher than that obtained from the two-stage dark fermentation/AD and single stage AD methods for biohydrogen from microalgae (Nannochloropsis Oceanica), respectively [116]. Metabolic engineering approach for selected strains is considered promising for improving the hydrogen kinetics and yields [32, 105]. Further research is required to develop viable solutions enhancing the hydrogen yield from microalgae.

3.2.5 Bio-oil (or bio-crude)

Microalgae can be suitable feedstocks for producing bio-oil (or "biocrude") via thermochemical conversion pathways, such as pyrolysis and hydrothermal liquefaction (HTL) [27]. As opposed to previous processes, which focused on converting specific components of algae, the thermochemical processes can convert the whole biomass into

bio-oil [25, 27, 28]. The HTL technology is considered promising as it does not require the drying of the harvested microalgal stream, which can be directly processed as wet (containing about 20-30% solids). The bio-oil produced can be stabilized and upgraded to various hydrocarbon biofuels, such as renewable gasoline, and jet fuel [25, 28, 117].

So far, various experimental works, under laboratory conditions, have demonstrated the viability of both pyrolysis and HTL as alternative methods to produce bio-oil from different microalgae species, see [118] and references therein.

Pyrolysis was performed using various microalgal feedstocks [27, 36]. However, since microalgae contain about 20-30% solids after the harvesting-dewatering stage, a thermal drying step is required prior to pyrolysis, because the process requires biomass feedstock with concentration of 80-90% solids [25, 28]. During the process, the biomass is rapidly heated to about 400-600°C in the absence of oxygen, under atmospheric pressure and then cooled within a few seconds [25, 36]. The main products of pyrolysis include: bio-oil (or "biocrude"), char, vapours and an aqueous phase upon condensation. The process is generally distinguished between fast and slow pyrolysis. Specifically, fast pyrolysis requires high biomass heating rates (e.g. 500 °C per min) and short vapour residence times (2-3 sec), to enhance bio-oil production. Slow pyrolysis uses lower biomass heating rates, e.g. 5-80 °C per min, and longer vapour residence time, namely 5-30 min, but has a reduced bio-oil yield and higher solid char yield [6].

With regard to fast pyrolysis of microalgae, the study of [28] reported that the mass distribution of the products amounted to 29.3% bio-oil, 13.6% char, 34.4% gases and 22.9% aqueous phase (reactor heated to 400 °C). As for bio-oil, experimental results indicate an average HHV of 38.7 MJ/kg, while an HHV of 25.4 and 7.3 MJ/kg were obtained for the char and gaseous fractions, respectively [28].

Heat for microalgae growth and processing can be recovered by capture of volatile species and by-products, such as char and gases released during the process, and then used for preheating the microalgae before pyrolysis [28]. By using volatiles and char for heat, imported energy can be avoided but useful energy yield from the original biomass will be about 90%.

The bio-oil produced can be directly combusted to generate electricity [25]. Alternatively, the bio-oil can be stabilized with the removal of the unwanted components (e.g. sulphur) by means of near critical liquid propane (as solvent in extraction columns), while keeping the viscosity at a desirable level [25]. Stabilized bio-oil can be further processed via hydrotreating/hydroprocessing to remove the excess of nitrogen and oxygen from the produced biofuel. To date, results on hydroprocessing of microalgae bio-oil are not yet available [28, 36].

The HTL conversion route appears promising for various strains [27, 118]. As mentioned before, this process can be viable for wet microalgae, avoiding the energy-intensive drying step needed in the case of pyrolysis [25, 28, 36, 119]. Prior to HTL, the biomass is pre-heated to approximately 150 °C for 30 min [119]. The HTL takes place in a reactor at temperatures of 250-370 °C and high pressure of 10-25 MPa in the presence of a catalyst such as zeolite or alkali salts (Na_2CO_3 or KOH), for at least 60 min [27, 119].

Similarly to pyrolysis, during HTL, the biomass slurry is converted into various fractions, including: bio-oil, which is the main energy carrier, solids, incondensable/light gases and an aqueous phase. The mass distribution of the HTL products depends on many factors, such as the chemical composition and solids content of microalgae, used catalyst, reaction temperature and retention time [36, 118]. A recent work on *Scenedesmus d.* grown in ORP indicates yields (by mass) of 37% bio-oil, 16% solids, 30% gases and 17% aqueous phase [28]. The produced bio-oil and gases (mainly CO_2) average HHV were found to be about 35 MJ/kg and 1.1 MJ/kg, respectively, independently from the microalgal strain used [28, 118]. The aqueous phase, which mainly contains dissolved organic carbon, ammonium and phosphite, might be ideally recycled to contribute to

supply the nutrients for the algal growth. Also, the catalyst and solid products can be separated from the bio-oil by centrifugation and reused within the system [28].

Similarly to the pyrolysis oil, the HTL bio-oil cannot be directly employed as transportation biofuel, but stabilization and hydroprocessing steps are required [25, 36]. As stated before, the aim of the hydroprocessing is to remove heteroatoms, such as nitrogen, oxygen and sulphur from the produced pyrolysis/HTL bio-oil.

A comparison of the main operational conditions of pyrolysis and HTL for bio-oil production from algae is given in Table 3.3. Microalgae bio-oil from both pyrolysis and HTL have similar composition in terms of nitrogen, sulphur and oxygen content [28].

In comparison with petroleum crude oil, microalgal bio-oil has higher oxygen and nitrogen content, see Table 3.4. The excess oxygen should be removed from microalgal biofuel to reach energetically favourable conditions during combustion [119].

The produced HTL bio-oil is expected to be used as renewable feedstock for co-refining in existing fossil based refineries [27, 119] Moreover, microalgal bio-oil can be integrated at various points in a refinery, depending on the quality of the biocrude and the desired biofuel product [27, 119].

Table 3.3. Comparison of the main operational conditions of pyrolysis and HTL for bio-oil production from algae, from [120].

	Pyrolysis	HTL
Temperature	400-600 °C	200-350 °C
Pressure	atmospheric pressure	50-200 bars
Water content of treated algal biomass	< 10%	80-90 %
Drying needed	Yes	No

Table 3.4. Average composition of HTL bio-oil recovered through processing of microalgae grown in WW effluent, in comparison with petroleum crude oil [117].

Constituent content (wt. %)	Microalgal bio-oil	Petroleum crude oil
Sulphur	0.5	1.42
Oxygen	5.5	0.1-1.5
Nitrogen	4.4	0.1-2
Carbon	78.7	83-87

4 Biofuels from algae: insights from LCA studies

4.1 Microalgal biodiesel pathways (via chemical processes)

The environmental performances of microalgal biodiesel pathways, especially in terms of GHG emissions and energy balances, were assessed in several studies with an LCA approach.

These studies normally include the analysis of the whole biodiesel supply chain, namely: microalgal production in ORP or PBR with lipids accumulation; harvesting-dewatering and extraction of lipids via chemical processing. The extracted lipids are then transformed into biodiesel by transesterification, similarly to the commercial biodiesel produced from other oil cops, e.g. soybeans, palm oil, canola etc., although this is not yet proven at large-scale for algal lipids [10].

To date, microalgal biodiesel is not commercially produced, mainly because of the high costs of production and extraction of lipids [121, 122]. The existing studies mostly analysed hypothetical scenarios based on a mix of assumed, modelled and/or experimental data extrapolated from laboratory results and/or pilot scale experiments, due to the lack of large-scale operational data.

The LCA studies found in the literature present different approaches and choices concerning the following aspects: i) functional unit; ii) system boundaries; iii) impact assessment modelling; iv) data quality and aggregation level etc.. Hence, it is not always possible or even meaningful to compare directly information available in the literature, as the results from modelled systems cannot be harmonized and normalised.

In this section, we analyse and interpret the energy and GHG emissions balances resulting from examined LCA studies. To this end, we focus on the specific parameters (namely, the life cycle inventory) that are essential to determine the performances of algal pathways, such as:

- microalgal growth rate;
- chemical composition and lipids yield;
- nutrients, CO_2 and water supplies;
- technology options and operational conditions for lipids extraction and conversion to fuel;
- co-products management.

Furthermore, we highlight the main features and assumptions of the modelling approaches found in the literature.

4.1.1 System definition of the reviewed LCA studies

The LCA studies reviewed are listed in Table A1 of the Annex. We selected the studies that evaluated the life-cycle energy and GHG emissions balances with reference to the biodiesel product, i.e. the functional unit (FU) is 1 MJ of biodiesel. These studies are based on an attributional LCA approach, therefore excluding potential market mediated effects induced by large-scale algal-biodiesel production [123, 124].

In many studies, the ORP system is considered as the most viable technology to grow microalgae for biofuels production [14, 24, 26, 67, 71, 74, 125]. Only one study [72] found the flat panel PBR to be more promising than the ORP due to the lower energy requirements; costs were not estimated, though. Hybrid configurations were also considered in some studies, including using a PBR for the inoculums preparation followed by microalgal growth in a ORP [71, 72, 126].

Figure 4.1 presents a schematic illustration of the material and energy input and output flows included in the main stages of a biodiesel production pathway [4, 107].

The main inputs from the technosphere for photoautotrphic microalgal cultivation in ORP/PBR include: nitrogen (N) and phosphorous (P) fertilizers and CO_2. Nutrients can be

supplied either by wastewater streams or synthetic fertilizers; CO_2 is usually obtained from flue gas streams or from other industrial processes, such as ammonia production.

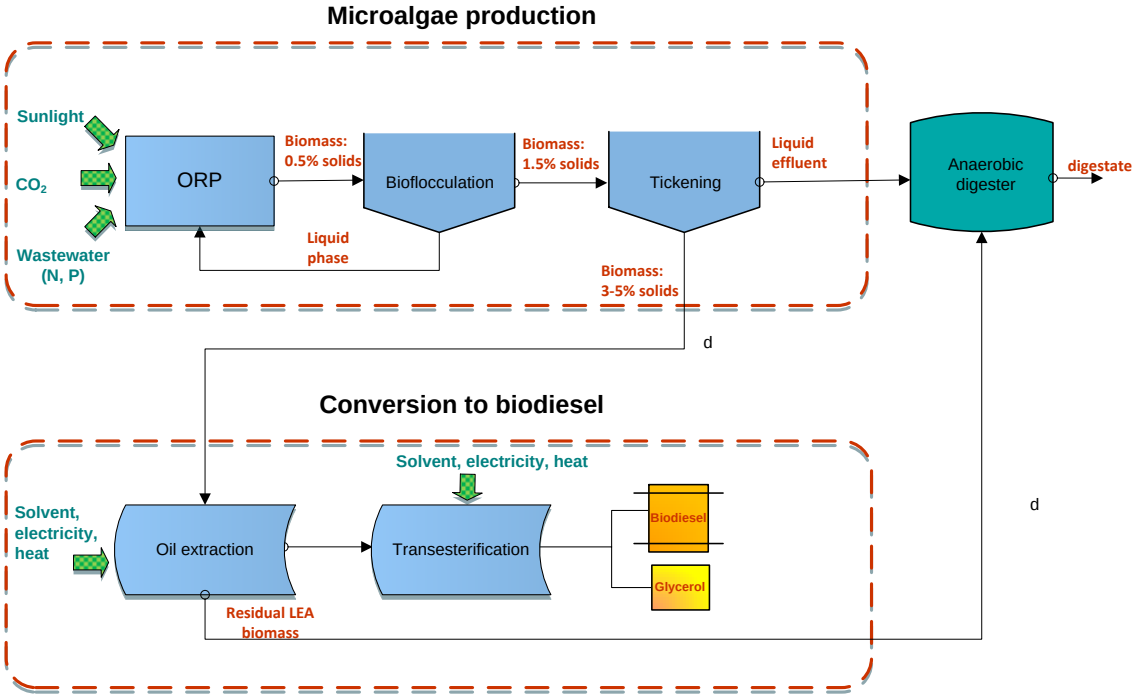

Figure 4.1. Main materials and energy inputs and outputs of biodiesel production from microalgae grown in ORP ("d" is the distance between the microalgal lipids production units and the conversion facilities), adapted from [14].

Many LCA studies have considered the growth of microalgae under nitrogen starvation with consequent enhancement of the accumulation of lipids in algal biomass [24, 26, 67, 125, 126]. Furthermore, the majority of LCA studies focused on converting the lipids into biodiesel via transesterification with methanol.

AD is the treatment most frequently considered for residual LEA biomass; the biogas produced is then combusted in a CHP engine to supply process heat and electricity [14, 72].

Two main co-products are produced in the process: glycerol from transesterification and LEA biomass. The treatment of these co-products is essential both for energy and GHG balances but it could also have an important economic impact on the overall plant.

Different system boundaries can be found for the analysis of the energy and GHG emissions balances, such as:

"cradle-to-gate", the production chain ends at the biodiesel plant gate [72];

"well-to-pump" or "strain to pump" including also the distribution of biodiesel to refuelling stations [71, 73, 74];

''well-to-wheels" or ''strain to wheels" including biofuel use in vehicles [14, 26, 73];

"cradle-to-grave" which also includes waste disposal [24, 125].

Table A.2 gives an overview of the main features of the microalgal cultivation systems, the area occupied by the modelled facilities, and site location for the microalgal biofuels systems proposed.

4.1.2 Life-cycle inventory analysis

4.1.2.1 Cultivation: inputs of nutrients, CO_2 and water

Depending on the selected species and growth conditions (in ORP or PBR systems), microalgae require appropriate amounts of N and P fertilizers, CO_2 and water.

Most studies assumed that the inputs of N and P were supplied by chemical fertilizers, such as urea and calcium/ammonium nitrate [24, 26, 67, 72-74, 125]. Usually, the source of CO_2 was considered to be the flue gases of a nearby power plant, with about 12.5% volume of CO_2 [14, 67, 72, 126]. CO_2 enriched air (2% CO_2) is also considered a possible carbon source [73].

It was commonly assumed that the growth media would need to provide amounts of nutrients and CO_2 equal to those contained in the microalgal biomass, on a stoichiometric basis. For this purpose, most studies used a generic molecular formula of microalgae [10]:

$$\mathbf{CO_{0.48}H_{1.83}N_{0.11}P_{0.01}} \qquad \text{Eq. 1}$$

Under N-Normal growth conditions, the inputs of N, P and CO_2 were estimated considering a ratio of C:N:P of 100:9:1. Under N-low growth conditions for lipids accumulation, the amounts of N, P and CO_2 were calculated with a ratio of C:N:P of about 100:3:1 [24, 26, 67, 71, 72, 125]. For these estimates, efficiencies of nutrients and CO_2 uptake between 85-100% for optimal microalgal growth rate were assumed [26, 61, 127]. It is worth considering that during the night and in poor weather (e.g. cloudy days), the algal biomass would slow down its growth rate and CO_2 uptake depending on the specific strain. Hence, CO_2 losses might occur in ORP systems and additional volume of gas may be needed to boost a higher rate of growth [128].

Alternatively, many LCA studies assumed that primarily treated WW streams could provide the required nutrients and water supplies for mixed algal and bacteria growth. Also in this case, an external CO_2 source is needed for optimal algal growth [14, 71]. This option is considered promising since it could generate an additional synergy in WWT plans where power, heat and CO_2 could be obtained from the AD of sewage sludge and algal biodiesel would constitute an additional high-value product.

In Table A.3, we provide an overview of the inputs of N and P fertilizers, CO_2 and the water required for producing 1 kg of microalgae as indicated in the literature. Most LCA studies did not include specific information on the make-up water requirements. The available estimations vary significantly, ranging between 1.3-4 and 239-373 litres per kg of dry microalgae on annual average [24, 26, 67]. The local temperature, the net solar radiation and the atmospheric water pressure may considerably affect the balance between the water supply and water evaporation for ORPs [24, 129, 130]. Furthermore, depending on the specific processes and conditions, the harvested water and liquid effluent of AD digestate can be partly recycled back to the ORP for algal growth, reducing the amount of make-up water that must be added to the system [26]. In case of high water consumption, there will be an increased electric energy for the water pumping and recirculation within the system. This emphasizes the need to consider the potential variability of the water demand for the algal growth in future LCA studies.

4.1.2.2 Biomass productivity and composition

Biomass productivity and biochemical composition are key parameters influencing the energy efficiency and GHG emissions of microalgal biodiesel pathways [26, 130]. Depending on the selected species and growth conditions, a large variability of these parameters is found in the literature.

Many sources report that N-starvation conditions can increase the lipids content of about 50-60%, for different species, with possible improvements of the overall performances of microalgal biodiesel pathways. However, total biomass productivities are expected to

also decrease by 28-50% in systems under N-low growth conditions, compared to the same systems under N-normal conditions [24, 67, 72].

Generally, higher growth rates were observed for microalgae grown in PBR compared to ORP [72].

A few LCA studies reported the detailed composition of the proposed microalgae strains, under different nutrients supply conditions [19, 65, 121]. This information is relevant for assessing the overall performance of the biodiesel value chain, especially in terms of materials and/or energy recovery potential from co-products management. The available datasets are collected in Table A.4.

4.1.2.3 Harvesting-dewatering technologies

Table A.5 summarizes the combination of harvesting-dewatering technologies considered in each study. Most authors assumed efficiencies of 90-95% for different harvesting-dewatering options [24, 26, 67, 72, 125, 126].

Overall, centrifugation is considered highly efficient for microalgal biomass dewatering. Though, it requires high energy inputs (see next section 4.1.3) and costs. Therefore, for a cost-efficient production of microalgal biomass, alternative, low-cost methods should be developed [24, 125]. Flocculation and settling have the potential to lower the energy inputs of the harvesting stage. However, the potential deployment of these technologies at large scale would require the use of large tanks and surface areas that need to be properly assessed in future LCA analysis [25].

4.1.2.4 Lipids extraction and conversion scenarios

As described in section 3.2.1, microalgal lipids can be extracted with hexane (organic solvent) on wet or dry microalgae.

Most LCA studies considered extraction of lipids from dry microalgal biomass using hexane as the most viable option. On the other hand, the thermal drying of microalgae is considered to be too energy-intensive and costly [24, 125]. To overcome this burden, some studies suggested the use of solar drying, assuming that no additional energy inputs would be required [71]. Solar drying has been utilized for many years in Asia to dry food-quality algae. However, the applicability of this method clearly depends on the climate conditions. Furthermore, this method would not be suitable for large-scale applications, mainly because of the large land area requirements and time needed to complete the drying process [69].

Some LCA studies considered the disruption of algal cells by homogenization at high pressure as an option for the wet extraction route [67, 71, 74]. Alternatively, the use of supercritical CO_2 ($ScCO_2$) was investigated as a means for lipids extraction from wet microalgal biomass [72, 74]. The $ScCO_2$ process includes compressing the CO_2 stream to high pressure for the selective dissolution of lipids content [14, 67, 74].

After extraction, a system for recovering and recycling back over 99% of the solvent must be considered for achieving a positive energy balance [67].

The conventional transesterification process with methanol, pure or mixed with chloroform, is required to convert the extracted microalgal oil to biodiesel (methyl esters) and glycerol, see Figure 4.1. Due to the lack of data, current LCA studies have assumed the transesterification process to be similar to that usually applied to soybean oil with an efficiency of 98-99% on mass basis [26, 72]. Specific results on microalgal oil conversion stages are required in future projects [125].

4.1.2.5 Life-cycle energy balance

We defined the net energy ratio (NER) as:

$$\mathbf{NER} = \frac{\mathbf{Non-renewable\ primary\ energy\ spent\ to\ produce\ the\ biofuel\ [MJ]}}{\mathbf{Energy\ contained\ in\ the\ biofuel\ [MJ]}} \quad \text{Eq. 2}$$

Estimates of NER values from literature are shown in Figure 4.2. The lower is the NER value, the more energy efficient is the pathway. If the NER is lower than 1, the system produces more energy than the fossil energy that was spent to produce the biofuel, of course excluding the solar energy embedded in the biomass. For comparison, the NER of fossil diesel pathway is also reported in Figure 4.2 [131].

Variations in the NER results mainly depend on the input needs and the process parameters underlying each stage, e.g.: energy consumption, productivity and lipids yield, system boundaries and how multifunctionality is solved, i.e. co-products allocation or substitution.

Most LCA studies included the AD as a treatment option of LEA biomass. In these configurations, energy is recovered through combustion of the biogas for internal uses, i.e. reduction of external energy input. In most cases it was assumed that glycerol was also fed to the AD plant. Alternatively, glycerol can be employed in the pharmaceutical industry or other markets. The liquid fraction of the digestate, instead, was assumed to be recirculated back to the ORP for nutrients recycling. In addition, it was often considered that the solid fraction of the digestate may displace synthetic fertilizers used in agriculture (displacement credits) [14, 24, 72, 74, 125]. Alternative options for the management of LEA biomass and glycerol are schematically illustrated in Figure 4.2.

LEA biomass was assumed to displace corn for ethanol production or fish feed in aquaculture, while glycerol would displace synthetic glycerol in pharmaceutical industry [71, 73]. The credits for the materials and energy carriers substituted by the co-products are subtracted from the overall NER of microalgal biodiesel pathways, as shown in Figure 4.2.

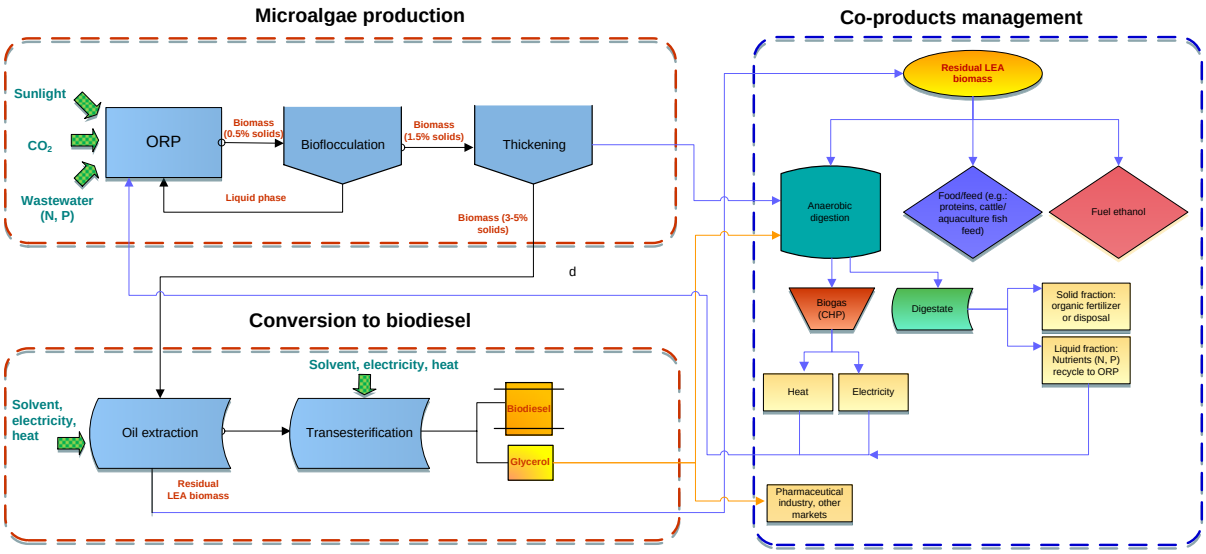

Figure 4.2. Main materials and energy inputs and outputs of biodiesel production from microalgae grown in ORP in combination with alternative options for the management of co-products, i.e. LEA biomass, glycerol and solid/liquid fractions of digestate.

Overall, for most pathways, without co-products credits, the energy consumed to produce microalgal biodiesel is higher than the energy in the biodiesel itself (i.e.

NER>1), and also higher than the primary energy demand associated to the production of fossil based diesel.

The results highlight that microalgae grown under N-low (starvation) conditions in ORP need lower energy input compared to microalgae grown under N-normal conditions. This can be associated to the lower fertilizers requirements and higher lipids yield that can be obtained under starvation conditions (e.g. *Chlorella v.* and *Scenedesmus d.*), see Table A3 and A4 in Annex [67, 125].

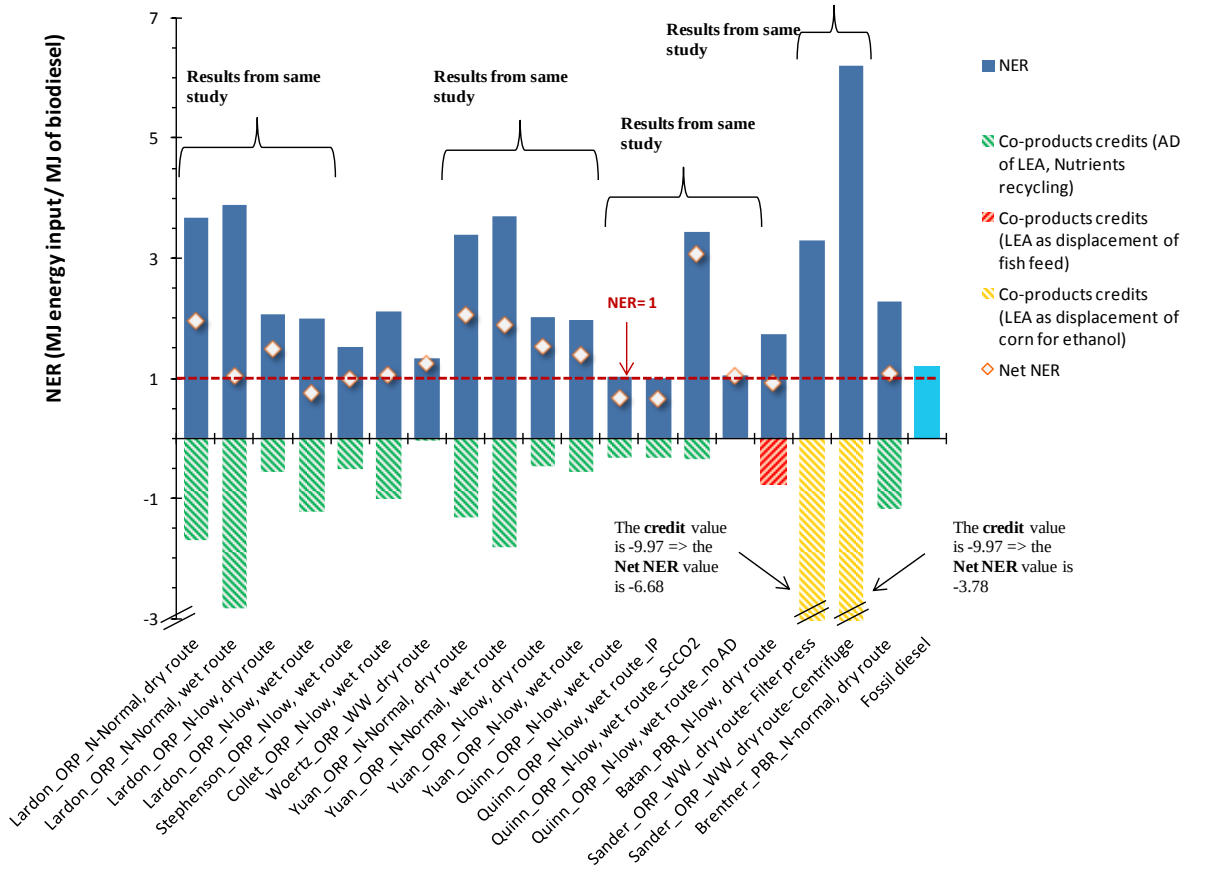

Figure 4.3. NER, namely the Life-cycle energy consumed/energy produced (NER <1 is desirable) of biodiesel pathways [14, 24, 26, 67, 71-74, 107, 125]. The effects of the integration of AD of LEA biomass and displacement credits for LEA as fish feed or corn for ethanol are shown as negative values. Diamonds displayed on each bar represent the net NER subtracting the credits from co-products management to the overall energy balance. For comparison, NER of WTW petroleum diesel production pathway is also indicated in the graph [131].

The wet lipids-extraction route requires less energy compared to the dry lipids-extraction route due to the lower heat and electricity requirements for the drying of the harvested biomass [24, 67, 74, 125].

The results of [71] show that the centrifugation process can lead to an increase of about 40% in the NER of biodiesel pathway compared to filtration.

Favourable net NER can be achieved for the wet lipids extraction on microalgae grown under N-normal and N-low conditions when considering the energy recovery from LEA biomass via AD, and displacement credits from solid digestate sold as fertilizer and from glycerol. It should be noted that the potential degradability and energy recovery from LEA biomass were determined either theoretically or experimentally at lab scale.

Therefore, these assumptions should be further investigated at larger scales and the uncertainty in the results should be considered in LCA studies.

The most favourable NER values were obtained by the studies in which large credits were assigned to LEA biomass, such as considering the displacement of corn for ethanol production (net NER of -3.29 and -6.19 MJ of energy input/MJ of biodiesel) [71].

These results highlight that the management of co-products is actually critical for evaluating the overall energy balance of biodiesel pathways. However, these credits are "numerically" essential to obtain positive NER for many pathways and these should be looked at critically. **The material substituted and the amounts of credits are modelling assumptions which may not accurately represent what may happen in reality.** Different considerations can be made on biogas production from LEA biomass. Electricity and heat produced from biogas can be re-used in the system, so they translate directly into energy savings. Only eventual credits for the sale of digestate as a source of agri-nutrients would fall back into the category of high uncertainty.

The outcomes of the analysis also underline that there is a need to decrease the energy (heat and electricity) and fertilizers consumptions to achieve favourable energy balance. To this end, the wet lipids extraction on microalgae grown under N-normal and N-low conditions seems to be a promising option. However, as already stated in previous sections, extraction methods for wet microalgal paste need to be fully developed and validated at a meaningful scale [24].

4.1.3 Life cycle GHG emissions

The GHG emissions balance of microalgal biodiesel pathways was calculated in several LCA studies and the results are summarized in Figure 4.4 [24, 26, 67, 71, 72, 107]. The estimates of GHG emissions are expressed in kg of $CO_{2\text{-eq}}$/MJ of biodiesel, based on the characterisation factors defined by the IPCC AR4 for a time horizon of 100 years (GWP-100) [132]. The results include the total GHG emissions due to the inputs for the production of 1 MJ of microalgae-based biodiesel (positive values) and the GHG emissions offsets (negative values) due to the co-products management (reduction of on-site heat and electricity requirements or substitution credits). The diamonds symbols displayed on each bar represent the net GHG values of biodiesel pathways. For comparison, the net GHG emissions of fossil derived diesel are also presented in Figure 4.4 [131].

The results fundamentally mimic the results of the energy balances presented in Figure 4.3. Large GHG emissions are found for most systems. The processes that contribute the most to the total are heat and electricity consumptions for the: microalgae cultivation, harvesting-dewatering, drying and lipids extraction and conversion to biodiesel [24, 26, 125].

Variations of the results depend on the specific technologies adopted, the system boundaries, modelling parameters and how multifunctionality is solved. The most impacting parameters are the fertilizers emissions and the process energy sources. Differences among the GHG emission factors of background systems considered (e.g. GHG emissions of the power generation system) may have a role in the differences among results.

The highest GHG emissions were found in the study of Lardon et al. [24], which modelled a cradle-to-grave system boundary including infrastructures and water treatment, see Table A1. The GHG emissions from all the pathways considered were significantly lowered when including the management of LEA biomass via AD. This is mainly due to the reduction of the external fossil-based energy requirements for microalgae biodiesel processing. In addition, the systems benefited from the credits for the displacement of synthetic fertilizers for the algal growth when digestate was recirculated to the ORP.

Large GHG credits were allocated to LEA biomass in case of displacement of corn for ethanol production [71]. LEA was assumed to have the same ethanol yield as wheat straw. Nevertheless, in the same study the net GHG emissions resulted still positive when centrifugation was used in the biomass harvesting-dewatering step, due to its high power requirements.

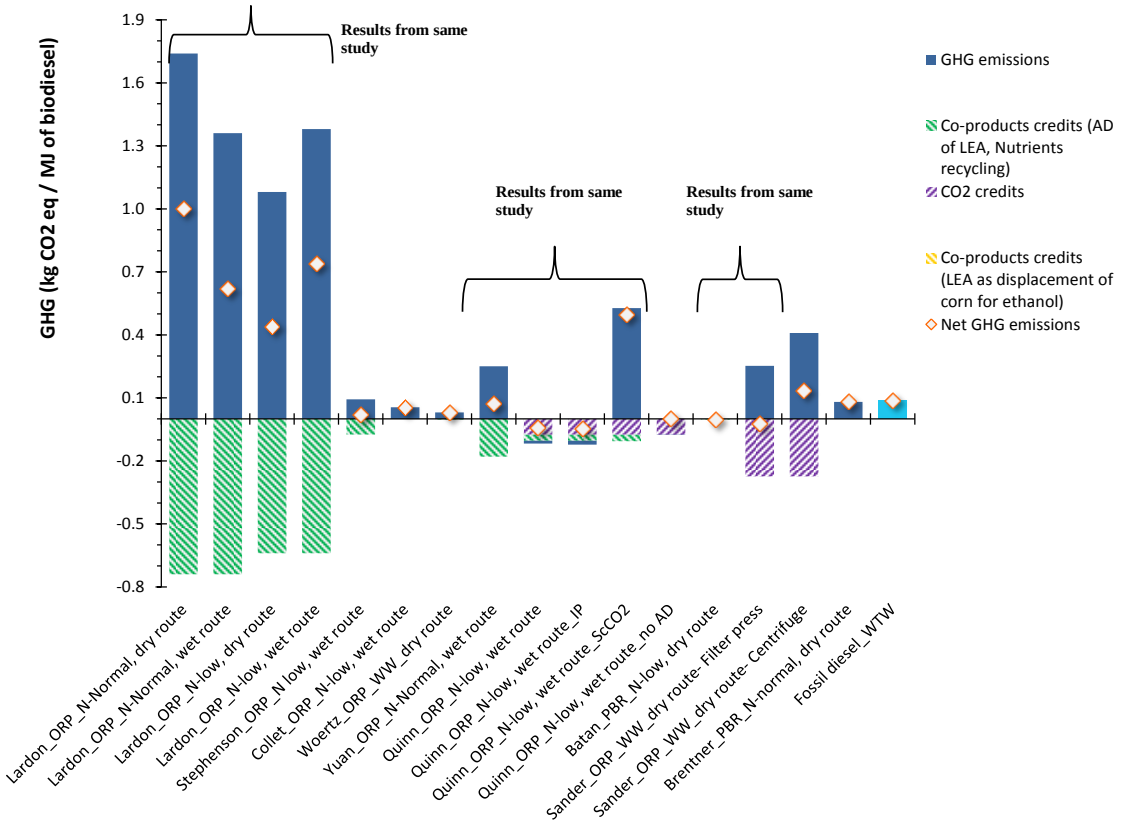

Figure 4.4. GHG emissions of microalgal biodiesel pathways referred to 1MJ biodiesel [24, 26, 67, 71, 72, 107]. The effects of the integration of AD of LEA biomass and displacement credits for LEA as fish feed or corn for ethanol are shown as negative values. Diamonds displayed on each bar represent the Net GHG emissions due to co-products management. For comparison, GHG emissions of WTT and WTW petroleum diesel production pathways are also indicated in the graph [131].

Another source of credits was considered in [73, 74]: credits were assigned for the CO_2 sequestration, from flue gas or atmospheric air, occurring via photosynthesis during microalgal growth. In the study of [71] similar CO_2 credits were assigned to the system with Well-to-Pump boundaries. The study of [70] assumed that algal growth is enhanced by CO_2 enriched atmospheric air (2% of CO_2 by volume), therefore assigning credits to the system for the net sequestration (only the net aggregated results are available).

However, it needs to be pointed out that we are dealing with the production of a fuel, thus the CO_2 fixed during photosynthesis will be re-emitted during the combustion of the biofuel in vehicle engines. Therefore, while it may be correct to subtract the Carbon fixation during the biomass growth, the same amount of CO_2 should be then considered as an emission at the point of combustion. However, since biogenic-CO_2 emissions from biofuels combustion are usually considered to be zero, assigning CO_2 credits to algal biodiesel production is highly misleading.

The low concentration of CO_2 in the atmosphere is a major impediment to the production of algal fuels at a substantial scale according to [68]. It is reported that a source of enriched CO_2 (e.g. from power plants or waste treatment processes) is necessary to cultivate microalgae with an improved productivity and oil yield per hectare [68].

Furthermore, it should be noted that the GHG emissions calculated in most studies do not account for some relevant sources of GHG emissions, such as direct N_2O emissions from the microalgal biodiesel pathways. Microalgae cultivation systems are aerobic environments, i.e. microalgae produce oxygen during growth, inhibiting the presence of denitrifying bacteria that can reduce the available nitrogen to N2O [73].

As for the indirect N_2O emissions, it is generally considered that 4% of the total N-input (urea) is volatilized as ammonia [67]. Ammonia emissions to the atmosphere generate indirect N_2O emissions that are often overlooked [132]. However, high uncertainty exist about ammonia volatilization rates from ORP, as concentrations of free ammonia in such systems can significantly vary depending on fertilizers composition; dissolved nitrate and oxygen concentration over dark periods; temperature and pH conditions. Empirical data from operational systems is lacking at present [133]. Considering the potential impacts of ammonia volatilization on other environmental areas of concern, e.g. acidification and eutrophication, as well as on the GHG balance of the biodiesel pathway, i.e. higher ammonia volatilization will imply larger consumptions of fertilizers, these phenomena need to be further investigated in future works.

4.2 Microalgal biocrude pathways via thermochemical methods

In this section, we describe the main features, assumptions, modelling approaches and results of the energy and GHG balances of microalgal biocrude pathways available in literature [25, 28].

The key parameters and processes affecting the results of the reviewed LCAs include: i) microalgal growth rate; ii) biochemical composition and lipids yield; iii) nutrients, CO_2 and water supplies; iv) technology options and operational conditions for biocrude production; v) biocrude stabilization (i.e. removal of unwanted components) and hydroprocessing for "drop-in" hydrocarbon biofuels or renewable diesel production; vi) co-products management.

4.2.1 System definition of the reviewed LCA studies

We reviewed the studies presented in Table A6 of the Annex. The studies evaluated the life-cycle energy and GHG emissions balances with reference to the final hydrocarbon biofuel or diesel product. The functional unit is MJ of biofuel.

The study of [25] investigated two pathways including the cultivation of mixed microalgal culture by using WW effluent and the cultivation of selected microalgal strain by using brackish water. While in [28], the pyrolysis and HTL experimental pathways, based on the results from lab-scale experiments, were analysed. In the latter, the authors also presented hypothetical industrial-scale system by assuming certain up-scaling improvements compared to the experimental set up, in terms of biomass yields and energy efficiencies of the biocrude production system.

All pathways considered the cultivation of microalgae in ORP; followed by harvesting-dewatering. In the case of pyrolysis, a thermal drying to 80-90% solids was necessary prior to the thermal treatment of the biomass, while HTL did not need any treatment prior to the catalytic hydroprocessing. The biocrude obtained is then stabilized and converted to "drop-in" hydrocarbon biofuel. A schematic overview of the main unit operations of microalgal biocrude production via pyrolysis or HTL is given in Figure 4.5 [25, 28, 119].

Co-products of the pathways include the aqueous phase and light gas stream (mainly containing nutrients and CO_2, respectively) that are expected to be recycled to the ORP for microalgal growth [25, 28]. Experimental tests have showed that the pyrolysis co-products, namely char, gasses and aqueous phase have mass yields of 13.6%, 34.3%, and 22.9% respectively [28]. Similarly, for the HTL pathway, the experimental yields of

bio-oil co-products, such as char, gasses and aqueous phase amounted to 16%, 30% and 17%, respectively [28].

In the LCA studies considered, char and non-condensable gasses were assumed to be combusted, with an efficiency of 85%, to recover process heat for internal uses [25, 28].

Different system boundaries were set for the analysis of the energy and GHG emissions balances, such as: "well-to-pump" in [28] and "well-to-wheels" in [25], see Table A6 of the annex for details on the examined microalgal biofuels systems. The energy requirements for transport and distribution of the final microalgal biofuel product to gas stations were also considered, based on the processes for soybean-derived biofuel [25, 28].

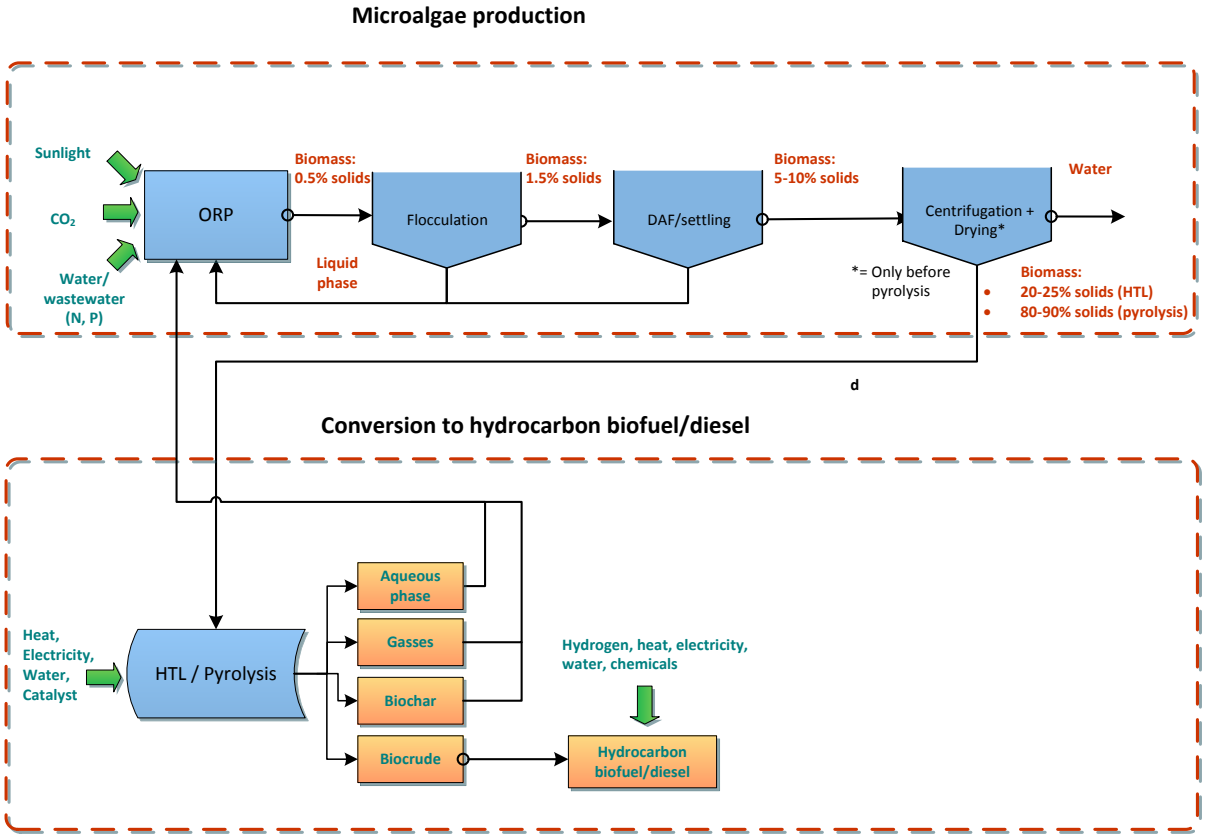

Figure 4.5. Main materials and energy inputs and outputs of biofuel production via pyrolysis or HTL from microalgae grown in ORP ("d" is the distance between the microalgal biomass production units and the conversion facilities), adapted from [25, 28].

4.2.2 Life Cycle inventory

4.2.2.1 Cultivation: inputs of nutrients, CO_2 and water

Available information on the main features and assumptions of the projected microalgal growth scenarios are reported in Table A7 [25, 28].

In [25], it is assumed that microalgal growth occur in a medium constituted of primarily treated WW, supplying nutrients and carbon sources, such as carbon-containing compounds and dissolved CO_2, necessary for algal growth. A second system was analysed where the cultivation of selected microalgal strains (Nannochloropsis sp.) occur in a brackish water medium, using the CO_2 from flue gas of a nearby-existing industry [25]. The study did not specify the inputs (N, P, CO_2 and energy) needed for optimal algal growth. Only aggregated information on the energy requirements of the cultivation stage were provided [25].

In [28], the cultivation of microalgae strain (*Scenedesmus d.*) takes place in a nutrients medium (BG-11 medium) for the experimental-scale system [28]. For the industrial-scale system, N and P fertilizers, i.e. urea and di-ammonium phosphate, were used as nutrients supplies. The Carbon source for the microalgal growth is assumed to be the atmospheric CO_2 for both scenarios. The amount of absorbed CO_2 was based on algal composition of 50% in Carbon, based on stoichiometric formula, as discussed in section 4.1.2.

Other sources have highlighted that, due to the low atmospheric CO_2 concentration (0.039% by volume), the CO_2 uptake from the atmosphere into the culture medium cannot not be sufficiently fast to rapidly grow a large concentration of algae [67, 81]. This emphasizes the uncertainty of this assumption.

The sources and estimates of the water supply for algal growth are not specified in the studies.

4.2.2.2 Biomass productivity and composition

Biomass productivity and lipids content of the microalgal biomass under different cultivation systems are presented in Table A8.

The system analysed in [25], which employed the WW effluent as a source of nutrients and CO_2 supply, was assumed to produce microalgae with a growth rate of 12 g/m^2/day and lipids content of 10% by mass (dry wt.). The values are about 50-65% lower than those assumed by [14] for similar scenarios (mixed algal biomass grown in WW effluent). These underline the uncertainty of key parameters, such as biomass yield and oil content used in LCA studies due to the lack of robust empirical data. The results also highlighted the need for a systematic sensitivity analysis of key parameters that could affect the LCA performance of the biofuel system under study.

The lab-scale system scenario investigated in assumed a biomass production of 6.5 g/m^2/day (on annual basis) [28]. It should be noted that an increased growth rates of about 100% was assumed in optimized industrial-scaled system [28].

4.2.3 Harvesting-dewatering technologies

A summary of the technology options considered in each microalgal biocrude pathways is presented in Table A9. In most scenarios, flocculation or bioflocculation systems, requiring minimum or no chemical inputs, followed by either settling tanks or DAF methods are proposed. The combination of these methods allowed to concentrate the biomass from less than 1% solids at the outset of the ORP to about 4-10% solids in the microalgal output stream, on a dry wt. base [25, 28].

For pyrolysis pathways, after the harvesting stage, centrifugation and thermal drying are necessary to achieve a solids concentration of 58% solids [25]. It is assumed that exhaust gases from the combustion of char and non-condensable gasses from pyrolysis contain enough heat to further dry the microalgae to 90% solids [25].

The study of [28] considered a system based on membrane filtration, leading to a slurry concentration of about 4% solids, in combination with centrifugation for a final concentration of 22% solids. The final drying stage, i.e. 90% solids, was performed by means of lyophilisation in the experimental system and through a rotary kiln operating with natural gas is used in the industrial scaled system [28].

In the HTL pathway, the wet microalgal slurry with about 20-25% solids, after centrifugation was supplied directly to the HTL reactor, avoiding the drying stage [28].

4.2.4 Life cycle energy balance

The NER results of microalgal biocrude pathways via pyrolysis or HTL, are reported in Figure 4.6 [25, 28]. Variations in the results depend on the: i) system boundaries; ii) assumptions underlying key process steps, e.g. biomass growth rate; efficiency of

harvesting-dewatering stages; ii) materials and energy inputs to biocrude recovery, stabilization and upgrading to final biofuel products.

For most pathways, without co-products credits, the energy consumed to produce microalgal biodiesel is higher than the energy in the biofuel itself, namely NER>1. The results also show that the NER of microalgal pathways via both pyrolysis and HTL can be significantly higher than that associated to the production of conventional diesel.

For the pyrolysis pathway with microalgae grown in WW [25], favourable NER values were obtained when large credits are assigned to the displacement of the biological nutrients removal (BNR) unit that typically is needed at the WWT plant for the effluent cleaning, see Figure 4.6. The conventional BNR is an energy intensive process due to high electricity and methanol consumptions to remove the nitrogen from the WW effluent before being discharged [25]. The BNR credits of the microalgal system amounted to about 1.16 kWh elec. and 0.31 kg Methanol per kg of grown algae. This is equivalent to an energy credit of about 2.2 MJ/MJ of microalgae bio-oil [25]. The technical viability of such an integrated system for nutrients removal from WW effluent and algal biomass growth must be further investigated at meaningful scales in future works.

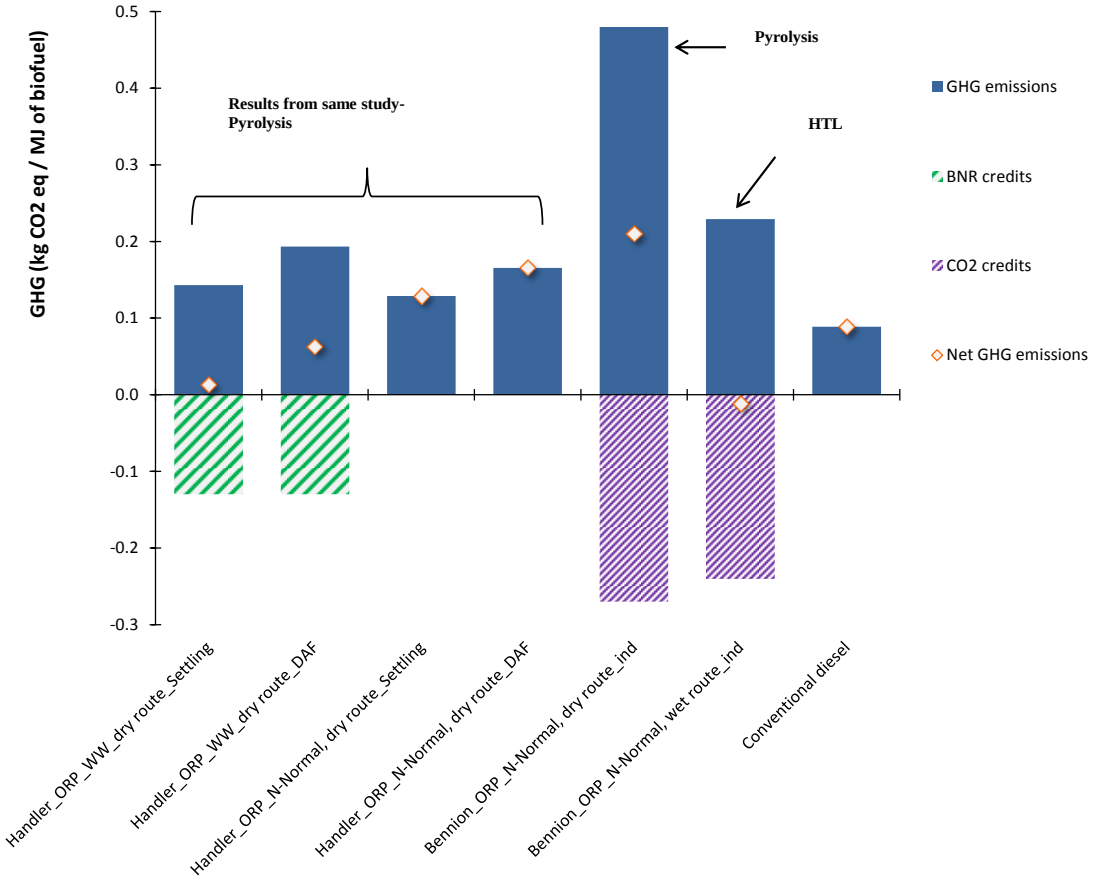

Figure 4.6. NER, , namely the Life-cycle energy consumed/energy produced (NER <1 is desirable) for the production of 1 MJ of microalgal biocrude via pyrolysis and HTL [25, 28]. The BNR displacement credits assigned to the microalgal growth system are shown as negative values. Diamonds displayed on each bar represent the net NER. For comparison, the NER of WTW petroleum diesel production pathway is also indicated in the graph [131].

On the other hand, the NER associated with other pyrolysis pathways show that they are not favourable if displacement credits are not assigned to the system. These results can be mainly ascribed to high heating demand of the dewatering-drying stage and the

operation of the pyrolysis reactor. The energy requirements of microalgal drying accounted for nearly half of the overall NER of the pyrolysis pathway, according to [25].

The most favourable NER was obtained for HTL pathway based on the modelling of an optimized system considering an improved microalgal yield and processes efficiency, i.e. reduced power for dewatering-drying techniques and bio-oil upgrading. Moreover, the optimized system assumed that part of the energy from the heat exchanger and burning of char and gasses co-products can be recovered and used within the system. The recovered energy amounts to about 10% of the overall energy demand for the HTL pathway [28]. The results highlight that the management of co-products is essential for achieving favourable energy balance of thermochemical pathways, i.e. reduction of on-site heat requirements for biomass drying.

4.2.5 Life cycle GHG emissions

The GHG balances of microalgal biocrude pathways are shown in Figure 4.7. The results were obtained from a "well-to-pump" or "well-to-wheels" system boundaries, as summarized in Table A.6 [25, 28].

In line with the results of the energy balances presented in Figure 4.6, the analyses of [25] show that higher GHG emissions than fossil-derived diesel are found for most systems [131].

With regard to the pyrolysis pathways, such as biomass grown in WW effluent with no input of fertilizers, the total emissions were significantly reduced by the large BNR credits given to the microalgal system [25]. For this scenario, the GHG emissions are reduced by about 30% (scenario: ORP_WW_dry route_DAF) to 85% (scenario: ORP_WW_dry route_settling) compared to the conventional diesel.

The lower emissions, of about 50 g of CO_2/MJ of biofuels, resulting for the harvesting by settling compared to the DAF can be ascribed to the lower energy requirements for compressing and delivering air to the microalgal culture [25].

The pyrolysis pathways (with either settling or DAF), which did not receive any displacement credits, showed higher emissions (of more than double) compared to those associated to conventional diesel. The results highlight that pyrolysis is not a promising strategy for microalgae biofuels production, even when the most favourable growth conditions and harvesting-dewatering approaches were assumed in the algal system. Much of the life cycle GHG emissions were associated to the biomass drying and power requirements of the pyrolysis reactor [25, 28]. The displacement potential of the conventional BNR units by using the microalgal growth system is a critical factor for achieving favourable results. The potential environmental and energetic benefits of such an integrated process need to be tested in operational microalgal production facilities at a meaningful scale.

In [28], the results, with "well-to-pump" boundaries, were reported only for the pyrolysis and HTL pathways of the industrial-scale system, such as improved microalgal yield and energy efficiency. The life-cycle GHG emissions associated to each of the sub-processes of the biofuel chain, such as the growth, dewatering, pyrolysis/HTL, bio-oil stabilization and hydrotreating, were presented in the study. Furthermore, the emissions were distinguished between process emissions, including heating, electrical and products consumption (i.e. nutrients demand, material losses and burning of co-products for energy recycling).

Overall, the results show that the HTL pathway has lower GHG emissions than pyrolysis, because of the avoidance of the emissions associated with the biomass drying.

In the pathways considered, the system received credits for the CO_2 uptake of the flue gas during growth. The pyrolysis pathway resulted in net emissions of 0.210 kg $CO_{2\text{-}eq}$/MJ of renewable biodiesel production, with higher impacts compared to conventional

diesel. The high emissions were mainly attributed to the drying of microalgae feedstock and heating of the reactor unit [28].

Considering the CO_2 credit, the HTL pathway showed net negative emissions of -0.011 kg $CO_{2\text{-eq}}$/MJ of biodiesel, with a reduction of about 30% compared to conventional diesel pathway. As mentioned previously, the CO_2 emissions of the systems should be accounted for at the point of combustion, such as in cars engine for example. The CO_2 fixed during photosynthesis will be re-emitted afterwards during combustion, unless the fuel produced is geologically stored.

Furthermore, the study assumed that the atmospheric CO_2 is fixed via photosynthesis for algal growth. As already mentioned, the CO_2 absorption from the atmosphere (with CO_2 concentration of 0.039% by volume) into the culture medium cannot not be sufficiently fast to rapidly grow a large concentration of algae [67, 81]. This emphasizes that the CO_2 credits, which are allocated to the microalgae-based systems, are wrongly assigned producing misleading results.

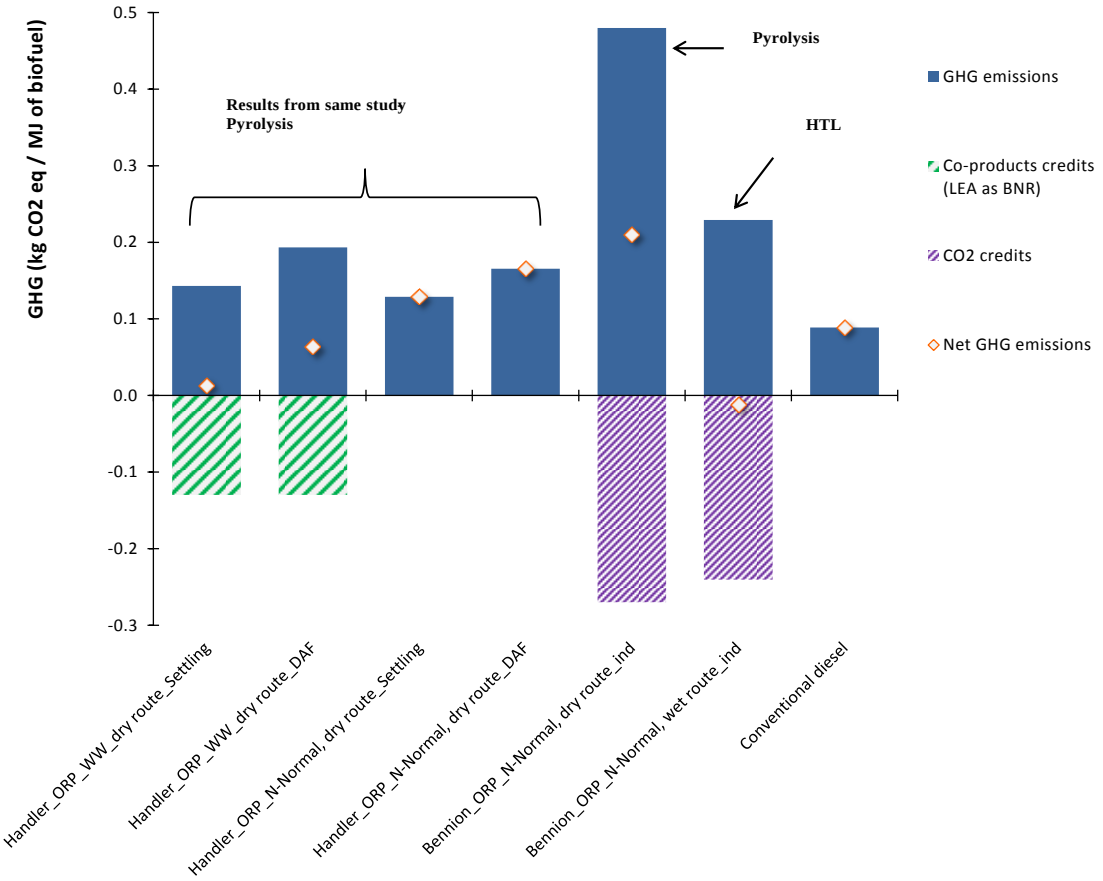

Figure 4.7. GHG emissions balance of 1MJ microalgal biodiesel pathways [25, 28]. The BNR displacement credits and CO_2 credits are shown as negative values. Diamonds displayed on each bar represent the Net GHG emissions. For comparison, GHG emissions of WTW petroleum diesel production pathways are also indicated in the graph [131].

4.3 Macroalgal biofuel pathways

4.3.1 System definition of the reviewed LCA studies

LCA studies on macroalgae as a source of biofuels are scarce in literature. This is mainly because of the early development stage of advanced biofuels from seaweeds [23, 97].

Many parameters, e.g. method of cultivation, yields of different species per hectare, time and method of harvest, etc. still lack of a proper assessment [97].

The only study of [134], which assessed the energy and GHG emissions balances of two hypothetical macroalgal biofuels scenarios, is based on literature and unpublished experimental data.

The scenarios analysed by [134] were:

- scenario 1 refers to the cultivation of a selected specie (*Laminaria digitata*) on "long line" systems in an offshore site in Denmark. After harvesting and mechanical pre-treatment (milling and grinding), the seaweed is anaerobically digested for biogas production (digestate is then used as fertilizer in agriculture);
- scenario 2 refers to the same cultivation, harvesting and pre-treatment systems of scenario 1. Then, the seaweed is converted to bioethanol via simultaneous saccharification and fermentation (SSF). The bioethanol produced is then distilled and blended with conventional gasoline. The residual biomass from SSF processes is used for biogas production via AD.

The initial seeding of macroalgal species under laboratory controlled conditions was also included in the analysis.

The FU is one tonne of dry wt. seaweeds cultivated and processed for biofuels production. Data on the composition (in terms of VS content) and biomethane potential (BMP) of the macroalgal specie considered were derived from experimental batch test performed at laboratory scale. The AD process is modelled by using data on the AD of WW sludge. Furthermore, due to the lack of data, the modelling scenarios of bioethanol production from seaweeds consider the results of bioethanol production from corn taken from Ecoinvent [52]. Flow diagrams involving the major steps of the different scenarios for bioethanol and/or biogas production from seaweed biomass are depicted in Figure 4.8.

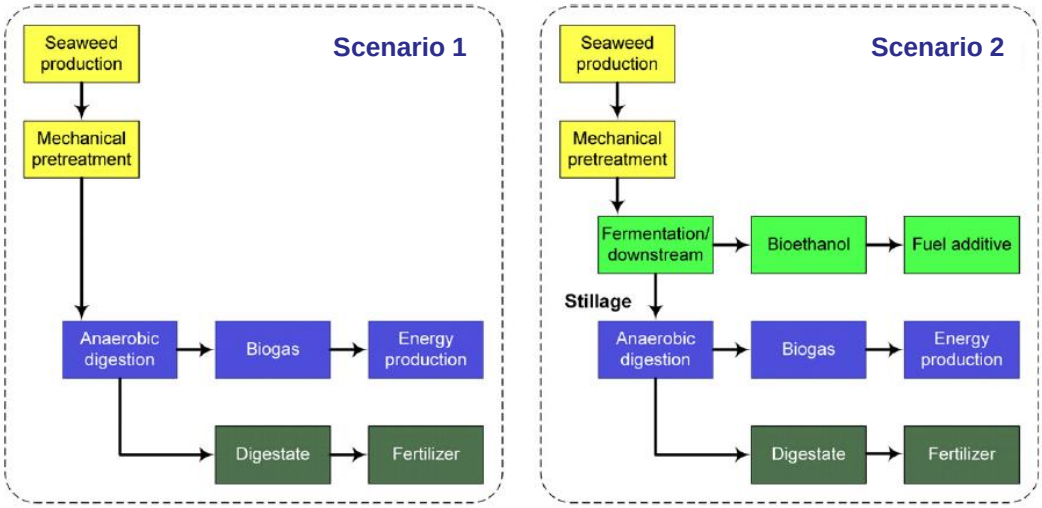

Figure 4.8. Main processes stages of biogas and bioethanol production from macroalgae grown at offshore site, from [134].

4.3.2 Life cycle inventory analysis

4.3.2.1 Cultivation and harvesting

Cultivation of macroalgae starts with the preparation of the seeds, which are fertile spores previously collected from the sea and their development on ropes, under controlled laboratory conditions, e.g. lighting and mixing.

Following, the production chain included the cultivation of macroalgae on a long-lines system at an offshore site in Denmark. A growing period of 4-6 months is expected to achieve the maximum biomass yield of the selected strain in the proposed cultivation system, i.e. 8.95 kg per meter of long-line [48].

The transportation needed for long-line development and harvesting was accomplished by means of a barge and observation and maintenance of the culture by means of a small skiff. The resulting diesel and petrol consumptions amounted to 30 litres each per tonne of dry wt. biomass.

The harvested macroalgae, containing about 10% solids, was cleaned and crushed by milling with a wet attritor.

4.3.2.2 Macroalgae processing to biofuels

In scenario 1, seaweeds slurry AD is carried out in a thermophilic system operating at the temperature of 52°C [134]. The estimated gross BMP of the selected strain (*Laminaria digitata*) amounted to 0.20 l CH_4/g VS under laboratory tests (VS content of 66.3% wt./wt.), in accordance with other studies [94, 135].

The biogas produced, i.e. about 220 m^3/tonne dry wt., was combusted in a CHP supplying heat and electricity to be used partially within the facility and exported to the grid.

The produced digestate was considered to displace synthetic fertilizer in agriculture (displacement credits by mass). This credit can be regarded as highly uncertain, due to the: i) nutrients characteristics; ii) logistic issues and iii) market-mediated effects.

In scenario 2, the pre-treated macroalgal biomass was used to produce bioethanol via a SSF process at the temperature of 32 °C. A bioethanol yield of 75 g/kg dry wt. biomass was assumed.

The production of bioethanol through the SSF process required the inputs of enzymes allowing the hydrolyzation of polysaccharides (cellulose and laminarin) to fermentable sugars [134]. The bioethanol is collected and sent to downstream unit operations, including stripping by a vapour stream and distillation, for biofuel concentration and upgrading (≥ 99.7% wt./wt.). The efficiency of downstream processes to recover the ethanol product from the fermenter effluent is assumed equal to 98%.

The produced residue from fermentation ("stillage") amounted to 891 g/kg of treated dry wt. seaweeds biomass. This residue was processed via AD, the gross biogas production was about 160 m^3/tonne dry wt.. The produced biogas is combusted in a CHP supplying heat and electricity to be used within the facility.

Also in this scenario, displacement credits were given to the digestate produced.

4.3.3 Life cycle energy balance

Estimates of energy inputs and outputs for the cultivation of one kg of dry wt. seaweeds for biogas and/or bioethanol production are presented in Figure 4.9. The scenarios considered include the inputs of electricity, heat, petrol and diesel for: i) macroalgal seeding and growth at offshore site; ii) harvesting; iii) mechanical pre-treatment; iv) biogas production for heat and electricity generation via AD (scenario 1); v) bioethanol and biogas production via fermentation and AD processes (scenario 2). For both scenarios, the impacts for infrastructures, e.g. hatchery, off-shore cultivation facilities, AD and ethanol plants, were not included in the analysis.

For scenario 1, the results show that the net energy consumption was higher than the energy contained in the produced biogas, with a NER above 1 (no net energy gain). This was mainly due to the heating of the seaweed biomass, from 8°C, which is the average annual temperature in Denmark, to 52°C, as required during the AD process.

The results also showed that the heat and electricity inputs of the digester accounted for about 47% of the total energy demand. As mentioned before, the heat requirements, i.e. 1.84 MJ/kg dry wt. biomass, were expected to be supplied by the combustion of the produced biogas [134].

For scenario 2, it was estimated that the electricity and heat expended for the fermentation process amounted to about 0.056 and 0.217 MJ/MJ of produced ethanol, respectively [134].

The results in Figure 4.9 indicate that significant consumptions of petrol and diesel are required in the both scenarios, due to the deployment of the long-lines culturing systems, as well as the observation and maintenance of the culture over the growth phase. These inputs account for 50 and 57% of the total energy demand in scenarios 1 and 2, respectively.

On the other hand, the estimated energy consumptions of the initial seeding steps, under controlled laboratory conditions, accounted for 5% of the total energy for seaweed production.

The results highlight that the cultivation and harvesting of seaweed in offshore site are the most intensive processes of the biofuel pathways [134]. The results underline the need of associating the macroalgal growth with existing platforms, such as aquaculture or wind systems, as an option to enhance the viability of macroalgae-based biofuel industry: potential shared infrastructures and maintenance with possible reduction of the investment and operational costs. The techno-economic assessment of such integrated systems should be evaluated in future works.

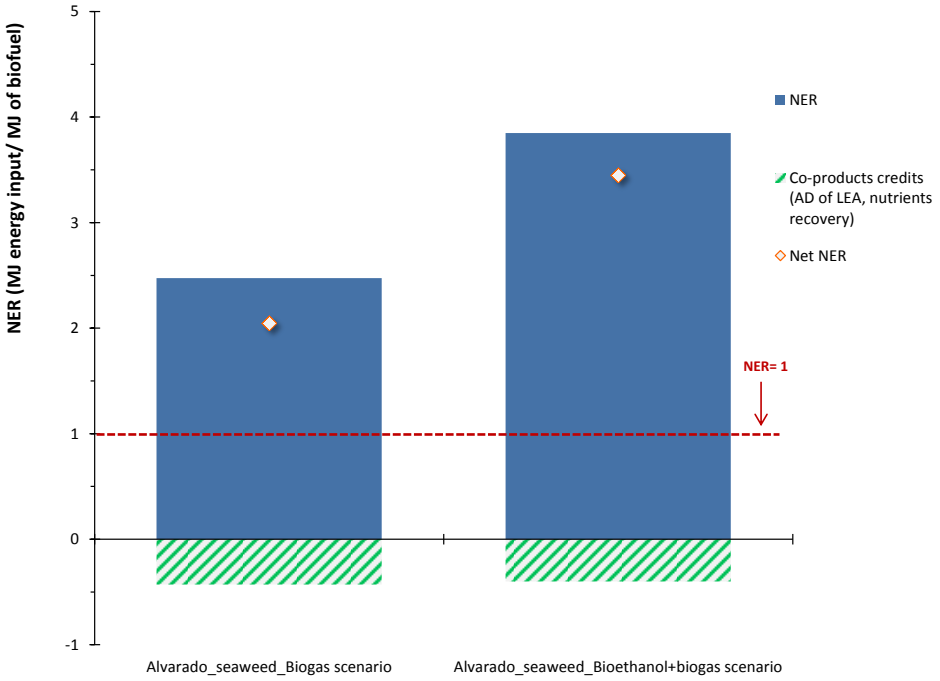

Figure 4.9. NER, namely the Life-cycle energy consumed/energy produced (NER <1 is desirable) for the production of biogas and/or bioethanol and biogas from seaweed via AD and fermentation in combination with AD, source: [134].

4.3.4 Life cycle GHG emissions

Estimates of the life-cycle GHG emissions, expressed in units of g of $CO_{2\text{-eq}}$/kg of seaweeds for biogas and bioethanol production are presented in Figure 4.10.

Similarly to the energy balance, the results highlight that the macroalgal production is regarded as the most energy intensive step of the whole biofuels chain. For both scenarios 1 and 2, significant emissions can be ascribed to the fossil diesel, petrol and electricity consumptions for the growing-out phase and maintenance of the seaweeds culture at the open sea site. Furthermore, for scenario 2 (bioethanol production), the downstream processes, for fermentation and bioethanol purification processes, also give a large contribution to the GHG emissions.

On the other hand, large credits were assigned, for the both the systems, to the bioethanol and/or electricity production from combustion of biogas in the CHP unit.

For scenario 1, savings can be obtained by considering the electricity recovery from biogas combustion that will be delivered to the grid, as well as the displacement of synthetic fertilizers through the digestate used in agriculture.

Similarly, for scenario 2, a reduction of external energy input was achieved by production of electricity with the AD of residual biomass deriving from the fermentation process (stillage). Displacement credits allocated to the digestate used in agriculture were also considered.

Furthermore, in scenario 2, GHG savings were given to the production of bioethanol as replacement of gasoline.

The results suggest that the BMP of both the whole seaweeds and the residual biomass originating from the fermentation process would have a large impact on the overall GHG emissions associated to the systems. To this regard, it should be noted that the allocation of the AD credits need to be further validated, as operational data from operational macroalgae-based systems, as well as SSF processes are not available yet.

The BMP of seaweeds can greatly vary depending on the composition of the substrate, which will in turn depend on season and culturing conditions, as well as operational AD parameters, such as HRT, OLR and temperature [97]. The actual methane yield and digester's performance need to be investigated in future research.

In the macroalgal bioethanol scenario, the actual electricity consumptions and bioethanol yields that can be obtained in operational fermentation plant can have large consequences on the environmental viability of the overall systems. However, in the reviewed study, the analysis of the input needs and parameters underlying the biofuels scenarios can be considered speculative, as operational data from large scale systems are not available yet. Furthermore, the LCA analysis was partly performed by using unpublished experimental data and personal communications that cannot be validated. The upstream and downstream fermentation processes for bioethanol production from seaweeds need to be investigated in future works to draw appropriate conclusions on scenarios efficiency and impacts.

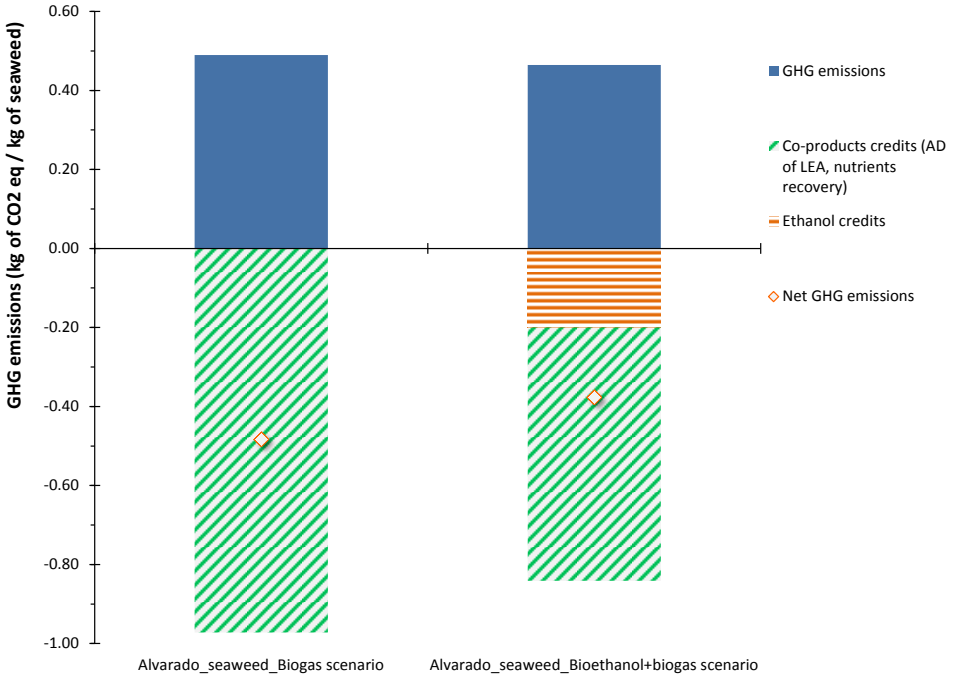

Figure 4.10. Total GHG emissions due to the inputs required for production of 1 kg of dry wt. seaweeds (positive values); GHG emissions offsets (negative values) due to the displacement credits given to the digestate, as well as to the ethanol and net electricity sent outside the system. The diamonds displayed on the bars represent the net GHG emissions resulting from the AD/ethanol credits considered within the biofuel pathways (GHG inputs-GHG offsets).

4.4 Summary of LCA results and needs for future works

In the sections above, we highlighted the main features, assumptions, modelling approaches and results of several algal biofuel pathways available in literature. These studies have assessed three main technological options:

i) Microalgal biodiesel via chemical processing;

ii) Microalgal biodiesel via thermochemical processing;

iii) Macroalgal biogas and bioethanol via biochemical processing.

For each of them, we focused on the life cycle inventory and on the parameters of the main process stages of the biofuel production chains, such as: i) biomass cultivation; ii) harvesting-dewatering; iii) lipids extraction and/or direct conversion to biofuel products; v) co-products management.

Concerning the data inventory and assumptions used in the LCA studies under review, we found that they have all considered hypothetical scenarios based on a mix of assumed, modelled and/or experimental data that have been extrapolated from laboratory results and/or pilot scale experiments. They are, therefore, not representing nor representative for actual plants. Furthermore, the analyses were partly performed using unpublished experimental data and personal contacts. Hence, relevant parameters, which could affect the LCA performance of the examined biofuel system, sometimes lack of transparency and calculations could not be reproduced.

About the methodological approaches adopted, we found that various LCA approaches were considered with regards to the:

- functional unit (FU);
- system boundaries;

- impacts assessment modelling;
- data quality and aggregation level.

Hence, it is not always possible to properly compare the information available in literature, as the results from modelled systems cannot be harmonized and normalised.

The large variations in the energy and GHG emissions balances depend, beside the specific technologies adopted, on the system boundaries, modelling parameters and how multifunctionality was solved. Especially the credits considered for co-products management play an essential role. Sensitivity analysis results are thus very important and these are missing for many pathways.

The main features and objectives of the reviewed LCA studies are presented in Table A1-A9 of the Annex. Major outcomes and considerations for the examined pathways are highlighted below.

i) Microalgal biodiesel pathways (via chemical processing):

- The **biomass productivity and lipids yield** of microalgal strains present large variability of values, depending on the inputs (nutrients, CO_2 sources, water supplies, solar radiation) and technology options (ORP and PBR) considered for microalgal growth. This also applies to microalgal biocrude pathway;
- Energy efficient and low-cost **harvesting-dewatering methods** need to be developed for a viable production of microalgal biomass. The potential deployment of promising technologies at large scale needs to be properly assessed in future LCA analyses including the infrastructures burden. This also applies also to microalgal biocrude pathway;
- The development of selected **high productivity and lipids-rich strains** is of critical importance for achieving viable microalgal biodiesel pathways, in terms of energy and GHG emissions balances;
- The development of **wet lipids extraction** on microalgae grown **under N-starvation** seems promising for achieving favourable energy and emissions balances of biodiesel pathways. However, extraction methods on wet microalgal paste need to be fully developed and validated at a meaningful scale;
- Appropriate management strategies of microalgal biodiesel **co-products**, such as LEA biomass and glycerol, are crucial for achieving favourable energy and emissions balances;
- The effective exploitation of LEA biomass via AD (energy savings) or as a displacement of corn for ethanol production or fish feed in aquaculture (displacement credits) should be validated in future work. The uncertainty in the results should be considered in LCA studies;
- If CO_2 credits are given to the systems during the algal growth, the CO_2 emissions from biodiesel combustion shall be accounted for at the point of combustion (this applies also to microalgal biodiesel via thermochemical processing). Since this is rarely considered, **CO_2 credits should not be assigned to algal biofuels**;
- The impacts of **ammonia volatilization rates** from ORP need to be investigated in future work, considering the potential effects on the GHG balance, i.e. higher ammonia volatilization will imply larger consumptions of fertilizers, and on other environmental areas of concern, e.g. acidification and eutrophication.

ii) Microalgal biodiesel pathways (via thermochemical processing):

- For most pathways, without considering co-products credits, the energy consumed to produce microalgal biofuels via pyrolysis and HTL is higher than the energy contained in the biofuels itself. The energy requirements for biocrude production can be significantly higher than those associated to the production of conventional diesel;
- Much of the life cycle **GHG emissions of pyrolysis pathways** are associated to the biomass drying and to the power requirements of the pyrolysis reactor. The total GHG emissions can be significantly lowered if large credits are assigned to

the algal cultivation system for the displacement of the **biological nutrients removal (BNR) unit that typically occur at the WWT plant (effluent cleaning);**

- The results highlight that the **system optimization, in terms of improved microalgal yield and processes efficiency, and management of co-products** are fundamental for achieving favourable energy balance of HTL pathways;
- Future efforts shall be made on developing continuous testing of different microalgal strains at a meaningful scale to validate the practical yield of algal biocrude and co-products generation for biofuel production.

iii) Macroalgal biogas and bioethanol pathways:

- The **production of macroalgae** can be identified as the most energy intensive step of the whole seaweed biofuel chain. Significant energy requirements and emissions can be ascribed to the **fossil diesel, petrol and electricity consumption** for the growing-out phase and maintenance of the seaweed culture at the open sea site.
- The available results underline the need of **integrating the macroalgal growth with existing platforms**, such as aquaculture or offshore wind systems, as an option to enhance the viability of macroalgae-based biofuel industry, thus, potentially sharing infrastructures and maintenance, with possible reduction of the investment and operational costs;
- The **methane yield** of both whole seaweeds and residual biomass from the fermentation process has a **large impact on the overall energy and GHG emissions** associated to possible macroalge processing to biogas and/or bioethanol systems;
- The BMP of seaweeds can vary greatly depending on their biochemical composition, which will in turn depend on season and cultivation conditions, as well as operational AD parameters, e.g. HRT, OLR and temperature. **The actual methane yield and digester's performance need to be investigated further in future research**.
- The upstream and downstream **fermentation processes** for bioethanol production from seaweeds need to be investigated in future works, especially in terms of actual electricity consumptions and bioethanol yields, to draw appropriate conclusions on scenarios efficiency and environmental impacts.

5 Conclusions and perspectives for future research

In the last decades, algae received considerable attention as possible feedstocks for biofuels and bioenergy contributing to the displacement of fossil fuels. However, to date, no commercial algae-based biofuel industry exists, mainly because of the lack of economically viable algae-to-biofuels production chains. Production of microalgal biofuels has not yet been demonstrated to be profitable and to date there seems to be no viable business case as commercial production of microalgal biomass remains a niche endeavour.

Furthermore, the exploitation of algae for biofuels production still presents significant challenges to overcome, from the technological, energetic and environmental points of view. These mainly include the identification and/or development of selected strains, technologies and plants configurations to enhance the energy returns of the overall biofuels production chain, while minimizing its related environmental impacts.

In **chapters 2 and 3**, we have reported on the current-status of the technological options for the production of algal biofuels from both macro- and microalgae species. We analyzed the main processing stages of the biofuel value chains, such as the: i) biomass cultivation; ii) harvesting-dewatering; iii) lipids extraction and/or direct conversion to biofuel products; v) co-products management.

In **chapter 4**, we have analysed and interpreted the energy and GHG emissions balances reported in LCA studies available in literature for various algal biofuels pathways. Furthermore, we have identified the main life cycle data inventory and the key parameters affecting the results.

Given the large variety of microalgal strains (40,000-100,000 species according to literature) and composition, the **selection of species** and **growth conditions** needs to be carefully designed in view of the proposed biofuels and/or bioproducts options. Positive algal features found in specific strains (e.g. improved yield under high light intensity; enhanced cells stability and resistance to infections) could be preferably combined in one ideal strain by genetic and metabolic manipulation of algae. The validation of effective algal growth rate and biomass characteristics based on large scale demonstrations is an essential priority of future work.

Considering the microscopic size and properties of microalgal strains, the development of **harvesting and dewatering** technologies represents a critical issue with respect to the energetic requirements and, accordingly, costs. At present, there is no comprehensive analysis on the deployment potential of optimized harvesting methods at large scale, in terms of technical viability, environmental impacts and cost effectiveness. Most of these aspects poses major technological challenges that have to be overcome in future work.

Several **technology options** have been proposed for algal processing to biofuels. However, these technologies have been tested only at the laboratory- or pilot-scale. As stated above, to date, algae for biofuel are still far from commercialization. The main barriers to large-scale deployment of both macro- and microalgae, include:

- High demands of key resources for algal growth, such as energy, nutrients, water and CO_2;
- Difficulty of maintaining selected species, e.g. with high productivity/lipids content, in outdoor culture;
- High capital and operational costs of production;
- High energy consumption associated with both the biomass production and its conversion to biofuels;
- The availability of land with suitable characteristics, i.e. climatic conditions and resource supply;
- Technical challenges of scaling up lab/pilot scale projects and cost effectiveness.

A major conclusion from existing **LCA analyses** of (experimental and/or projected) algal biofuels pathways is that the **biomass productivity and lipids yield** of microalgal

strains present large variability of values, depending on the inputs, e.g. nutrients, CO_2 sources, water supplies and solar radiation, as well as technology options, e.g. ORP and PBR. The effects of the variation of key parameters on the LCA results should be properly addressed in a broad sensitivity analysis to provide a spectrum of model outputs deriving from possible configurations of the same pathway. The results may offer a valuable contribution to ultimately identify research priorities, optimal system configurations and potential environmental risks.

Energy efficient and low-cost **harvesting-dewatering methods** shall be developed for a viable production of microalgal biomass. The potential deployment of promising technologies at large scale needs to be accurately assessed in future LCA analyses considering the infrastructures burden.

The **management of co-products** derived from algal biofuels pathways, e.g. residual LEA biomass, glycerol, solid and liquid digestate, is crucial for achieving favourable energy and emission balances of biodiesel pathways. The most favourable NER values are obtained when large credits were assigned to LEA biomass, such as the displacement of corn for ethanol production or fish feed. However, these credits are "numerically" essential to obtain positive NER for many pathways but they should be looked at critically. The material substituted and the amounts of credits are merely modelling assumptions which may not accurately represent what may happen in reality. With regards to effective biogas production from LEA biomass, the re-use of electricity and heat within the system translates directly into energy savings. Demonstrate the AD performance of the extracted co-products (LEA biomass) of conversions pathways is thus a key priority of future works.

Alternative strategies of potential utilization of co-products as animal or fish feed, and fermentation for bioethanol production may present valuable opportunities for cost reduction and performance improvement. However, any potential co-products employment option needs to be carefully assessed, also in terms of market-mediated impacts and related uncertainty.

Recently, there has been a major interest in the development and validation of integrated processes employing biomass power plant supplied with agricultural residues to produce CO_2, heat and electricity for the growth of algae in ORP, in combination with WWT effluent as the growth medium. Algae can be further processed into biogas that is then upgraded to compressed natural gas to be used in vehicle engines. Efforts are also made in validating the efficacy of extraction and use of co-products and intermediates to generate CO_2 and electricity to meet the needs of the algal plants. Techno-economic challenges and environmental impacts of algae-to fuels strategies need to be properly assessed (comprehensive impacts assessment lack at the present) before implementing systems integration strategies leading to the deployment of the algal biofuels industry.

The development of macroalgal biofuels in combination with existing platforms, such as aquaculture or wind systems, is a challenging approach for enhancing the potential of future commercialization and costs reduction (potentially shared infrastructures and maintenance, with possible reduction of the investment and operational costs). Furthermore, costs offsets may be derived by the ability of algae to effectively use the WWT effluent as a source of water and nutrients (substitution of BNR occurring at the WWT plant). Integrating the processes of WWT and algal biomass production may potentially constitute a more cost effective approach than traditional WWT operations. Future efforts shall be focused on the effective assessment and possible implementation of viable technologies aiming at: i) coupling algal biofuel production with low-cost CO_2 from flue gas, waste heat and wastewater sources; ii) implementing viable bio-refining schemes for the production of high value-added products in combination with biofuels products.

References

[1] M. F. Jonna, J. James, and A. Simon, "Carbon footprint of seaweed as a biofuel," *The Crown Estate: Marine Estate Research Report,* 2012.

[2] P. Gerbens-Leenes, L. Xu, G. Vries, and A. Hoekstra, "The blue water footprint and land use of biofuels from algae," *Water Resources Research,* vol. 50, pp. 8549-8563, 2014.

[3] L. Marelli, M. Padella, R. Edwards, A. Moro, M. Kousoulidou, J. Giuntoli, D. Baxter, V. Vorkapic, A. Agostini, A. O'Connell, L. Lonza, and L. Garcia-Lledo, "The impact of biofuels on transport and the environment, and their connection with agricultural development in Europe. Directorate-General for internal policies policy department b: structural and cohesion policies transport and tourism. http://www.europarl.europa.eu/RegData/etudes/STUD/2015/513991/IPOL_STU%282015%29513991_EN.pdf," ed, 2015.

[4] "Renewable Energy Directive 2009–28-EC Annex 1 " *European Commission,* 2012.

[5] A. Agostini, J. Giuntoli, and A. Boulamanti, "Carbon accounting of forest bioenergy," 2013.

[6] EC, "Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. A policy framework for climate and energy in the period from 2020 to 2030.http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:52014DC0015," 2014.

[7] "DIRECTIVE (EU) 2015/1513 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 9 September 2015 amending Directive 98/70/EC relating to the quality of petrol and diesel fuels and amending Directive 2009/28/EC on the promotion of the use of energy from renewable sources."

[8] V. Patil, K.-Q. Tran, and H. R. Giselrød, "Towards sustainable production of biofuels from microalgae," *International journal of molecular sciences,* vol. 9, pp. 1188-1195, 2008.

[9] J. A. Alic, "Biofuel battles: Politics, policy, and the pentagon," *Energy Research & Social Science,* vol. 10, pp. 10-18, 2015.

[10] Y. Chisti, "Biodiesel from microalgae," *Biotechnology Advances,* vol. 25, pp. 294-306, 2007.

[11] L. Brennan and P. Owende, "Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products," *Renewable and Sustainable Energy Reviews,* vol. 14, pp. 557-577, 2010.

[12] A. Darzins, P. Pienkos, and L. Edye, "Current status and potential for algal biofuels production," *A report to IEA Bioenergy Task,* vol. 39, 2010.

[13] B. Zhao, J. Ma, Q. Zhao, L. Laurens, E. Jarvis, S. Chen, and C. Frear, "Efficient anaerobic digestion of whole microalgae and lipid-extracted microalgae residues for methane energy production," *Bioresource technology,* vol. 161, pp. 423-430, 2014.

[14] I. C. Woertz, Benemann, John R, Du, Niu, Unnasch, Stefan, Mendola, Dominick, Mitchell, B Greg, Lundquist, Tryg, "Life Cycle GHG Emissions from Microalgal Biodiesel–a CA-GREET Model," *Environmental science & technology,* vol. 48, pp. 6060-6068, 2014.

[15] S. F. S. Hena, S. Tabassum, "Cultivation of algae consortium in a dairy farm wastewater for biodiesel production," *Water Resources and Industry,* vol. *In press, corrected proof*, 2015.

[16] B. S. Sturm and S. L. Lamer, "An energy evaluation of coupling nutrient removal from wastewater with algal biomass production," *Applied Energy,* vol. 88, pp. 3499-3506, 2011.

[17] W. Zhou, P. Chen, M. Min, X. Ma, J. Wang, R. Griffith, F. Hussain, P. Peng, Q. Xie, and Y. Li, "Environment-enhancing algal biofuel production using wastewaters," *Renewable and Sustainable Energy Reviews,* vol. 36, pp. 256-269, 2014.

[18] L. Zhu, "Biorefinery as a promising approach to promote microalgae industry: An innovative framework," *Renewable and Sustainable Energy Reviews,* vol. 41, pp. 1376-1384, 2015.
[19] M. Rizwan, J. H. Lee, and R. Gani, "Optimal design of microalgae-based biorefinery: Economics, opportunities and challenges," *Applied Energy,* vol. 150, pp. 69-79, 2015.
[20] J. Trivedi, M. Aila, D. P. Bangwal, S. Kaul, and M. O. Garg, "Algae based biorefinery—How to make sense?," *Renewable and Sustainable Energy Reviews,* vol. 47, pp. 295-307, 2015.
[21] "Algae-based biofuels: A Review of Challenges and Opportunities for Developing Countries" Food and Agriculture Organization of the United Nations2010.
[22] C. Enzing, M. Ploeg, M. Barbosa, L. Sijtsma, M. Vigani, C. Parisi, and E. R. Cerezo, *Microalgae-based products for the food and feed sector: an outlook for Europe*: Publications Office, 2014.
[23] T. Burton, H. Lyons, Y. Lerat, M. Stanley, and M. B. Rasmussen, "A review of the potential of marine algae as a source of biofuel in Ireland," Dublin: Sustainable Energy Ireland-SEI2009.
[24] L. Lardon, A. Hélias, B. Sialve, J.-P. Steyer, and O. Bernard, "Life-cycle assessment of biodiesel production from microalgae," *Environmental science & technology,* vol. 43, pp. 6475-6481, 2009.
[25] R. M. Handler, D. R. Shonnard, T. N. Kalnes, and F. S. Lupton, "Life cycle assessment of algal biofuels: Influence of feedstock cultivation systems and conversion platforms," *Algal Research,* 2014.
[26] A. L. Stephenson, E. Kazamia, J. S. Dennis, C. J. Howe, S. A. Scott, and A. G. Smith, "Life-cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors," *Energy & Fuels,* vol. 24, pp. 4062-4077, 2010.
[27] D. López Barreiro, W. Prins, F. Ronsse, and W. Brilman, "Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects," *Biomass and Bioenergy,* vol. 53, pp. 113-127, 2013.
[28] E. P. Bennion, D. M. Ginosar, J. Moses, F. Agblevor, and J. C. Quinn, "Lifecycle assessment of microalgae to biofuel: Comparison of thermochemical processing pathways," *Applied Energy,* 2015.
[29] R. J. Radmer and B. C. Parker, "Commercial applications of algae: opportunities and constraints," *Journal of applied phycology,* vol. 6, pp. 93-98, 1994.
[30] Q. Hu, Sommerfeld, Milton, Jarvis, Eric, Ghirardi, Maria, Posewitz, Matthew, Seibert, Michael, Darzins, Al, "Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances," *The Plant Journal,* vol. 54, pp. 621-639, 2008.
[31] K. Chojnacka and A. Noworyta, "Evaluation of *Spirulina sp.* growth in photoautotrophic, heterotrophic and mixotrophic cultures," *Enzyme and microbial technology,* vol. 34, pp. 461-465, 2004.
[32] U.S.DOE, "National Algal Biofuels Technology Roadmap. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program.," Workshop and Roadmap sponsored by the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Office of the Biomass Program 2010.
[33] S.-K. Kim, "Handbook of Marine Macroalgae: Biotechnology and Applied Phycology," *John Wiley & Sons, 04/nov/2011*.
[34] A. S. Carlsson, J. B. van Beilen, R. Möller and D. Clayton, "Micro- and macro-algae: utility for industrial applications. Outputs from the EPOBIO project September (http://www.biofuelstp.eu/downloads/epobio_aquatic_report.pdf), 2007.
[35] R. Rajkumar, Z. Yaakob, and M. S. Takriff, "Potential of the micro and macro algae for biofuel production: A brief review," *BioResources,* vol. 9, pp. 1606-1633, 2013.

[36] F. Murphy, G. Devlin, R. Deverell, and K. McDonnell, "Biofuel production in ireland—An approach to 2020 targets with a focus on algal biomass," *Energies,* vol. 6, pp. 6391-6412, 2013.
[37] E. Allen, J. Browne, S. Hynes, and J. Murphy, "The potential of algae blooms to produce renewable gaseous fuel," *Waste management,* vol. 33, pp. 2425-2433, 2013.
[38] N. Neveux, M. Magnusson, T. Maschmeyer, R. Nys, and N. A. Paul, "Comparing the potential production and value of high-energy liquid fuels and protein from marine and freshwater macroalgae," *GCB Bioenergy,* 2014.
[39] S. Kraan, "Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production," *Mitigation and Adaptation Strategies for Global Change,* vol. 18, pp. 27-46, 2013.
[40] C. Rosenberg and J. Ramus, "Ecological growth strategies in the seaweeds Gracilaria foliifera (Rhodophyceae) and Ulva sp.(Chlorophyceae): soluble nitrogen and reserve carbohydrates," *Marine Biology,* vol. 66, pp. 251-259, 1982.
[41] M. Mohammadi, H. Tajik, and P. Hajeb, "Nutritional composition of seaweeds from the Northern Persian Gulf," *Iranian Journal of Fisheries Sciences,* vol. 12, pp. 232-240, 2013.
[42] G. Jard, H. Marfaing, H. Carrère, J. Delgenes, J. Steyer, and C. Dumas, "French Brittany macroalgae screening: composition and methane potential for potential alternative sources of energy and products," *Bioresource technology,* vol. 144, pp. 492-498, 2013.
[43] J. W. van Hal, W. Huijgen, and A. López-Contreras, "Opportunities and challenges for seaweed in the biobased economy," *Trends in biotechnology,* vol. 32, pp. 231-233, 2014.
[44] K. A. Jung, Lim, Seong-Rin'Kim, Yoori, Park, Jong Moon, "Potentials of macroalgae as feedstocks for biorefinery," *Bioresource technology,* vol. 135, pp. 182-190, 2013.
[45] A. D. Hughes, M. S. Kelly, K. D. Black, and M. S. Stanley, "Biogas from Macroalgae: is it time to revisit the idea," *Biotechnol Biofuels,* vol. 5, p. 86, 2012.
[46] N. Wei, J. Quarterman, and Y.-S. Jin, "Marine macroalgae: an untapped resource for producing fuels and chemicals," *Trends in biotechnology,* vol. 31, pp. 70-77, 2013.
[47] S. van den Burg, M. Stuiver, F. Veenstra, P. Bikker, A. L. Contreras, A. Palstra, J. Broeze, H. Jansen, R. Jak, and A. Gerritsen, "A Triple P review of the feasibility of sustainable offshore seaweed production in the North Sea," ed: Wageningen UR, 2013.
[48] J. Langlois, J. F. Sassi, G. Jard, J. P. Steyer, J. P. Delgenes, and A. Hélias, "Life cycle assessment of biomethane from offshore-cultivated seaweed," *Biofuels, Bioproducts and Biorefining,* vol. 6, pp. 387-404, 2012.
[49] IEA-Bioenergy-Task39, "Algae as a Feedstock for Biofuels. An Assessment of the Current Status and Potential for Algal Biofuels Production," September, 2011.
[50] A. Fudholi, K. Sopian, M. Y. Othman, and M. H. Ruslan, "Energy and exergy analyses of solar drying system of red seaweed," *Energy and Buildings,* vol. 68, pp. 121-129, 2014.
[51] I. Mazarrasa, Y. S. Olsen, E. Mayol, N. Marbà, and C. M. Duarte, "Global unbalance in seaweed production, research effort and biotechnology markets," *Biotechnology Advances,* 2014.
[52] D. Aitken, C. Bulboa, A. Godoy-Faundez, J. L. Turrion-Gomez, and B. Antizar-Ladislao, "Life cycle assessment of macroalgae cultivation and processing for biofuel production," *Journal of Cleaner Production,* vol. 75, pp. 45-56, 2014.
[53] H. Chen, D. Zhou, G. Luo, S. Zhang, and J. Chen, "Macroalgae for biofuels production: Progress and perspectives," *Renewable and Sustainable Energy Reviews,* vol. 47, pp. 427-437, 2015.
[54] A. Richmond, *Handbook of microalgal culture: biotechnology and applied phycology*: John Wiley & Sons, 2008.

[55] P. G. Falkowski, M. E. Katz, A. H. Knoll, A. Quigg, J. A. Raven, O. Schofield, and F. Taylor, "The evolution of modern eukaryotic phytoplankton," *science,* vol. 305, pp. 354-360, 2004.
[56] F. Alam, A. Date, R. Rasjidin, S. Mobin, H. Moria, and A. Baqui, "Biofuel from algae-Is it a viable alternative?," *Procedia Engineering,* vol. 49, pp. 221-227, 2012.
[57] Y. Chisti and J. Yan, "Energy from algae: Current status and future trends: Algal biofuels–A status report," *Applied Energy,* vol. 88, pp. 3277-3279, 2011.
[58] Y. Chisti, "Fuels from microalgae," *Biofuels,* vol. 1, pp. 233-235, 2010.
[59] R. Garofalo, "Algae and aquatic biomass for a sustainable production of 2nd generation biofuels," *AquaFUELs-Taxonomy, Biology and Biotechnology,* pp. 1-258, 2009.
[60] T. M. Mata, A. A. Martins, and N. S. Caetano, "Microalgae for biodiesel production and other applications: a review," *Renewable and Sustainable Energy Reviews,* vol. 14, pp. 217-232, 2010.
[61] L. Rodolfi, G. Chini Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, and M. R. Tredici, "Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor," *Biotechnology and Bioengineering,* vol. 102, pp. 100-112, 2009.
[62] A. O. Alabi, "Microalgae technologies & processes for biofuels/bioenergy production in British Columbia: Current Technologies, Suitability & Barriers to Implementation," January 14, 2009 2009.
[63] L. Zhu, E. Hiltunen, E. Antila, J. Zhong, Z. Yuan, and Z. Wang, "Microalgal biofuels: Flexible bioenergies for sustainable development," *Renewable and Sustainable Energy Reviews,* vol. 30, pp. 1035-1046, 2014.
[64] P. Biller, B. K. Sharma, B. Kunwar, and A. B. Ross, "Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae," *Fuel,* vol. 159, pp. 197-205, 2015.
[65] J. P. Maity, J. Bundschuh, C.-Y. Chen, and P. Bhattacharya, "Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives–A mini review," *Energy,* 2014.
[66] M. Tredici, *Mass Production of Microalgae: Photobioreactors. In Handbook of Microalgal Culture: Biotechnology and Applied Phycology*: Edited by Amos Richmond. Blackwell Science, 2004.
[67] J. Yuan, A. Kendall, and Y. Zhang, "Mass balance and life cycle assessment of biodiesel from microalgae incorporated with nutrient recycling options and technology uncertainties," *GCB Bioenergy,* 2014.
[68] D. Klein-Marcuschamer, Y. Chisti, J. R. Benemann, and D. Lewis, "A matter of detail: assessing the true potential of microalgal biofuels," *Biotechnology and Bioengineering,* vol. 110, pp. 2317-2322, 2013.
[69] J. Ferrell, "National Algal Biofuels Technology Roadmap. A technology roadmap resulting from the National Algal Biofuels Workshop December 9-10, 2008 College Park, Maryland," May 2010 2010.
[70] M. K. Lam and K. T. Lee, "Microalgae biofuels: a critical review of issues, problems and the way forward," *Biotechnology Advances,* vol. 30, pp. 673-690, 2012.
[71] K. Sander and G. S. Murthy, "Life cycle analysis of algae biodiesel," *The International Journal of Life Cycle Assessment,* vol. 15, pp. 704-714, 2010.
[72] L. B. Brentner, M. J. Eckelman, and J. B. Zimmerman, "Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel," *Environmental science & technology,* vol. 45, pp. 7060-7067, 2011.
[73] L. Batan, J. Quinn, B. Willson, and T. Bradley, "Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae," *Environmental science & technology,* vol. 44, pp. 7975-7980, 2010.

[74] J. C. Quinn, T. G. Smith, C. M. Downes, and C. Quinn, "Microalgae to biofuels lifecycle assessment—multiple pathway evaluation," *Algal Research,* vol. 4, pp. 116-122, 2014.
[75] R. Slade, Bauen, Ausilio, "Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects," *Biomass and Bioenergy,* vol. 53, pp. 29-38, 2013.
[76] H. Chen, T. Qiu, J. Rong, C. He, and Q. Wang, "Microalgal biofuel revisited: An informatics-based analysis of developments to date and future prospects," *Applied Energy,* vol. 155, pp. 585-598, 2015.
[77] A. Singh and S. I. Olsen, "A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels," *Applied Energy,* vol. 88, pp. 3548-3555, 2011.
[78] A. I. Barros, A. L. Gonçalves, M. Simões, and J. C. Pires, "Harvesting techniques applied to microalgae: A review," *Renewable and Sustainable Energy Reviews,* vol. 41, pp. 1489-1500, 2015.
[79] S. Tedesco, T. Marrero Barroso, and A. Olabi, "Optimization of mechanical pre-treatment of Laminariaceae spp. biomass-derived biogas," *Renewable Energy,* vol. 62, pp. 527-534, 2014.
[80] C. Nurra, E. Clavero, J. Salvadó, and C. Torras, "Vibrating membrane filtration as improved technology for microalgae dewatering," *Bioresource technology,* vol. 157, pp. 247-253, 2014.
[81] Y. Chisti, "Constraints to commercialization of algal fuels," *Journal of biotechnology,* vol. 167, pp. 201-214, 2013.
[82] A. Kumar, X. Yuan, A. K. Sahu, J. Dewulf, S. J. Ergas, and H. Van Langenhove, "A hollow fiber membrane photo-bioreactor for CO2 sequestration from combustion gas coupled with wastewater treatment: a process engineering approach," *Journal of chemical technology and biotechnology,* vol. 85, pp. 387-394, 2010.
[83] A. Jacob, A. Xia, and J. D. Murphy, "A perspective on gaseous biofuel production from micro-algae generated from CO2 from a coal-fired power plant," *Applied Energy,* vol. 148, pp. 396-402, 2015.
[84] R. P. John, G. Anisha, K. M. Nampoothiri, and A. Pandey, "Micro and macroalgal biomass: a renewable source for bioethanol," *Bioresource technology,* vol. 102, pp. 186-193, 2011.
[85] M. Martín and I. E. Grossmann, "Design of an optimal process for enhanced production of bioethanol and biodiesel from algae oil via glycerol fermentation," *Applied Energy,* vol. 135, pp. 108-114, 2014.
[86] P. Morand and X. Briand, "Anaerobic digestion of Ulva sp. 2. Study of Ulva degradation and methanisation of liquefaction juices," *Journal of applied phycology,* vol. 11, pp. 164-177, 1999.
[87] X. Briand and P. Morand, "Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation," *Journal of applied phycology,* vol. 9, pp. 511-524, 1997.
[88] D. Waart, in *Biogas from seaweeds. In Morand P., Schulte E.H., Aquatic primary biomass (Marine Macroalgae): Biomass conversion, removal and use of nutrients. Proceedings of the 1st Workshop of yhe COST48 SubGroup 3. L'Houmeau, France.* , 1988.
[89] M. Orlandini, Favretto, L., "Utilisation of macroalgae in Italy for pollution abatement and as source of energy and chemicals. Proceeding of the 3rd COST 48-subgroup, Crema-L'Homeau, CNRS-IFREMER, France, pp. 25–28.," 1987.
[90] D. Wise, D. Augenstein, and J. Ryther, "Methane fermentation of aquatic biomass," *Resource Recovery and Conservation,* vol. 4, pp. 217-237, 1979.
[91] J. F. Hanssen, Indergaard, Mentz, Østgaard, Kjetill, Bævre, Olav Arne, Pedersen, Tor A Jensen, Arne, "Anaerobic digestion of *Laminaria spp.* and *Ascophyllum nodosum* and application of end products," *Biomass,* vol. 14, pp. 1-13, 1987.
[92] K. Østgaard, M. Indergaard, S. Markussen, S. H. Knutsen, and A. Jensen, "Carbohydrate degradation and methane production during fermentation of

Laminaria saccharina (Laminariales, Phaeophyceae)," *Journal of applied phycology,* vol. 5, pp. 333-342, 1993.
[93] A. Vergara-Fernández, G. Vargas, N. Alarcón, and A. Velasco, "Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system," *Biomass and Bioenergy,* vol. 32, pp. 338-344, 2008.
[94] E. Allen, D. M. Wall, C. Herrmann, A. Xia, and J. D. Murphy, "What is the gross energy yield of third generation gaseous biofuel sourced from seaweed?," *Energy,* 2015.
[95] M. Montingelli, S. Tedesco, and A. Olabi, "Biogas production from algal biomass: A review," *Renewable and Sustainable Energy Reviews,* vol. 43, pp. 961-972, 2015.
[96] M. Alvarado-Morales, I. B. Gunnarsson, I. A. Fotidis, E. Vasilakou, G. Lyberatos, and I. Angelidaki, "Laminaria digitata as a potential carbon source for succinic acid and bioenergy production in a biorefinery perspective," *Algal Research,* vol. 9, pp. 126-132, 2015.
[97] J. D. Murphy, B. Drosg, E. Allen, J. Jerney, A. Xia, and C. Herrmann, "A perspective on algal biogas," *IEA Bioenergy,* 2015.
[98] G. Hansson, "Methane production from marine, green macro-algae," *Resources and conservation,* vol. 8, pp. 185-194, 1983.
[99] M. Ras, L. Lardon, S. Bruno, N. Bernet, and J.-P. Steyer, "Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*," *Bioresource technology,* vol. 102, pp. 200-206, 2011.
[100] R. Samson and A. LeDuy, "Improved performance of anaerobic digestion of *Spirulina maxima* algal biomass by addition of carbon-rich wastes," *Biotechnology letters,* vol. 5, pp. 677-682, 1983.
[101] B. Carpentier, "Digestion anaérobie de la biomasse algale: les résidus de l'extraction de l'acid alginique, les ulves de mare e verte. Th. 3'eme cycle: Algologie. Univ. Paris 6, France: 101 pp.," 1986.
[102] A. Bruhn, J. Dahl, H. B. Nielsen, L. Nikolaisen, M. B. Rasmussen, S. Markager, B. Olesen, C. Arias, and P. D. Jensen, "Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion," *Bioresource technology,* vol. 102, pp. 2595-2604, 2011.
[103] K. T. Bird, D. P. Chynoweth, and D. E. Jerger, "Effects of marine algal proximate composition on methane yields," *Journal of applied phycology,* vol. 2, pp. 207-213, 1990.
[104] N.-J. Kim, Li, Hui, Jung, Kwonsu, Chang, Ho Nam, Lee, Pyung Cheon, "Ethanol production from marine algal hydrolysates using *Escherichia coli* KO11," *Bioresource technology,* vol. 102, pp. 7466-7469, 2011.
[105] C. Sambusiti, M. Bellucci, A. Zabaniotou, L. Beneduce, and F. Monlau, "Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: A comprehensive review," *Renewable and Sustainable Energy Reviews,* vol. 44, pp. 20-36, 2015.
[106] D. L. Barreiro, M. Beck, U. Hornung, F. Ronsse, A. Kruse, and W. Prins, "Suitability of hydrothermal liquefaction as a conversion route to produce biofuels from macroalgae," *Algal Research,* vol. 11, pp. 234-241, 2015.
[107] P. Collet, A. Hélias, L. Lardon, J.-P. Steyer, and O. Bernard, "Recommendations for Life Cycle Assessment of algal fuels," *Applied Energy*.
[108] H. K. Reddy, T. Muppaneni, Y. Sun, Y. Li, S. Ponnusamy, P. D. Patil, P. Dailey, T. Schaub, F. O. Holguin, and B. Dungan, "Subcritical water extraction of lipids from wet algae for biodiesel production," *Fuel,* vol. 133, pp. 73-81, 2014.
[109] E. A. Ehimen, S. Connaughton, Z. Sun, and G. C. Carrington, "Energy recovery from lipid extracted, transesterified and glycerol codigested microalgae biomass," *GCB Bioenergy,* vol. 1, pp. 371-381, 2009.
[110] M. Dębowski, M. Zieliński, A. Grala, and M. Dudek, "Algae biomass as an alternative substrate in biogas production technologies—Review," *Renewable and Sustainable Energy Reviews,* vol. 27, pp. 596-604, 2013.

[111] B. Sialve, N. Bernet, and O. Bernard, "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable," *Biotechnology Advances,* vol. 27, pp. 409-416, 2009.
[112] L. Mendez, A. Mahdy, M. Ballesteros, and C. González-Fernández, "Chlorella vulgaris vs cyanobacterial biomasses: Comparison in terms of biomass productivity and biogas yield," *Energy Conversion and Management,* vol. 92, pp. 137-142, 2015.
[113] H. M. El-Mashad, "Biomethane and Ethanol Production Potential of *Spirulina platensis* Algae and Enzymatically Saccharified Switchgrass," *Biochemical Engineering Journal,* 2015.
[114] E. Ehimen, Z. Sun, C. Carrington, E. Birch, and J. Eaton-Rye, "Anaerobic digestion of microalgae residues resulting from the biodiesel production process," *Applied Energy,* vol. 88, pp. 3454-3463, 2011.
[115] J. Giuntoli, A. Agostini, R. Edwards, and L. Marelli, "Solid and gaseous bioenergy pathways: input values and GHG emissions," *Report EUR,* vol. 26696, 2014.
[116] A. Xia, C. Herrmann, and J. D. Murphy, "How do we optimize third-generation algal biofuels?," *Biofuels, Bioproducts and Biorefining,* 2015.
[117] M.-O. P. Fortier, G. W. Roberts, S. M. Stagg-Williams, and B. S. Sturm, "Life cycle assessment of bio-jet fuel from hydrothermal liquefaction of microalgae," *Applied Energy,* vol. 122, pp. 73-82, 2014.
[118] E. D. Frank, A. Elgowainy, J. Han, and Z. Wang, "Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae," *Mitigation and Adaptation Strategies for Global Change,* vol. 18, pp. 137-158, 2013.
[119] X. Liu, B. Saydah, P. Eranki, L. M. Colosi, B. Greg Mitchell, J. Rhodes, and A. F. Clarens, "Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction," *Bioresource technology,* vol. 148, pp. 163-171, 2013.
[120] C. Hognon, F. Delrue, J. Texier, M. Grateau, S. Thiery, H. Miller, and A. Roubaud, "Comparison of pyrolysis and hydrothermal liquefaction of Chlamydomonas reinhardtii. Growth studies on the recovered hydrothermal aqueous phase," *Biomass and Bioenergy,* vol. 73, pp. 23-31, 2015.
[121] R. H. Wijffels and J. M. Barbosa, "An Outlook on Microalgal Biofuels," *Science,* vol. Vol. 329 pp. pp. 796-799 2010.
[122] FAO, "Aquatic Biofuels Working Group- Algae-based biofuels: A Review of Challenges and Opportunities for Developing Countries. FAO Environment and Natural Resources Service Series, No. 33 – FAO, Rome 2009. ," 2009.
[123] R. J. Plevin, M. A. Delucchi, and F. Creutzig, "Using attributional life cycle assessment to estimate climate-change mitigation benefits misleads policy makers," *Journal of Industrial Ecology,* vol. 18, pp. 73-83, 2014.
[124] I. Handbook, "General guide for Life Cycle Assessment," *Joint Research Centre (JRC) of the European Commission,* 2010.
[125] P. Collet, L. Lardon, A. Hélias, S. Bricout, I. Lombaert-Valot, B. Perrier, O. Lépine, J.-P. Steyer, and O. Bernard, "Biodiesel from microalgae–Life cycle assessment and recommendations for potential improvements," *Renewable Energy,* vol. 71, pp. 525-533, 2014.
[126] J. C. Quinn, A. Hanif, S. Sharvelle, and T. H. Bradley, "Microalgae to biofuels: Life cycle impacts of methane production of anaerobically digested lipid extracted algae," *Bioresource technology,* vol. 171, pp. 37-43, 2014.
[127] I. Rawat, R. Ranjith Kumar, T. Mutanda, and F. Bux, "Biodiesel from microalgae: A critical evaluation from laboratory to large scale production," *Applied Energy,* vol. 103, pp. 444-467, 2013.
[128] P. K. Campbell, T. Beer, and D. Batten, "Life cycle assessment of biodiesel production from microalgae in ponds," *Bioresource technology,* vol. 102, pp. 50-56, 2011.
[129] T. J. Lundquist, I. C. Woertz, N. Quinn, and J. R. Benemann, "A realistic technology and engineering assessment of algae biofuel production," 2010.

[130] R. M. Handler, C. E. Canter, T. N. Kalnes, F. S. Lupton, O. Kholiqov, D. R. Shonnard, and P. Blowers, "Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts," *Algal Research,* vol. 1, pp. 83-92, 2012.
[131] R. Edwards, J.-F. Larivé, D. Rickeard, and W. Weindorf, "Description, results and input data per pathway. WELL-TO-WHEELS ANALYSIS OF FUTURE AUTOMOTIVE FUELS AND POWERTRAINS IN THE EUROPEAN CONTEXT (Appendix 4 - Version 4a.)," *JRC Technical report,* 2013.
[132] S. Solomon, *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* vol. 4: Cambridge University Press, 2007.
[133] F. Gao, L. Gao, D. Zhang, N. Ye, S. Chen, and D. Li, "Enhanced hydrolysis of Macrocystis pyrifera by integrated hydroxyl radicals and hot water pretreatment," *Bioresource technology,* 2014.
[134] M. Alvarado-Morales, A. Boldrin, D. B. Karakashev, S. L. Holdt, I. Angelidaki, and T. Astrup, "Life cycle assessment of biofuel production from brown seaweed in Nordic conditions," *Bioresource technology,* vol. 129, pp. 92-99, 2013.
[135] C. Vanegas and J. Bartlett, "Green energy from marine algae: biogas production and composition from the anaerobic digestion of Irish seaweed species," *Environmental technology,* vol. 34, pp. 2277-2283, 2013.

List of abbreviations

AD	Anaerobic digestion
BMP	Biomethane potential
BNR	Biological nutrients removal
CHP	Combined heat and power
CSTR	Continuously stirred tank reactor
DAF	Dissolved Air Flotation
FU	Functional unit
GHG	Greenhouse gas
ha	Hectare
HRT	Hydraulic retention time
HTL	hydrothermal liquefaction
K	Potassium
LCA	Life Cycle Assessment
LEA biomass	Lipids-extracted algal biomass
MF	Microfiltration
N	Nitrogen
NER	Net energy ratio
OLR	Organic loading rate
ORP	Open Raceway Pond
P	Phosphorous
PBR	Photobioreactor
SSF	simultaneous saccharification and fermentation
TSS	Total suspended solids
UF	Ultrafiltration
VS	Volatile solids
wt.	Weight
WTT	Well-to-tank
WTW	Well-to-wheels
WW	Wastewater
WWT	Wastewater treatment

Annex

Table A.1. Summary of the main objectives, systems boundaries and functional unit (FU) of previous LCA studies on microalgae-to-biodiesel processing and modelling scenarios (ordered from the oldest to the most recent).

Reference	Objectives of the LCA study	System boundaries	FU
Lardon et al. [24]	Comparison of two fertilization conditions for microalgae growth, i.e. N sufficient supply and N starvation supply (i.e. based on the approximate molecular formula of microalgae biomass and its protein content); comparison of dry and wet methods for extraction of lipids from microalgae.	"Cradle to grave" analysis of the biodiesel production system; "Cradle-to-combustion" analysis of the fuel in a diesel engine.	1 kg of biodiesel
Batan et al. [73]	Analysis of PBR system for microalgae growth, on average yearly basis. The microalgae growth model was based on the results from pilot scale reactor, including the recycling of growth media but not that of Nitrogen from lipids-extracted biomass.	"Well to pump" analysis through GREET 1.8c model, including growth stage; dewater (via centrifugation); oil extraction and conversion to biodiesel; transport and distribution of biodiesel to consumer pumping station. Energy required for construction of ORP and PBR excluded.	1 MJ of biodiesel
Sander et al. [71]	Comparison of microalgae dewatering methods, i.e. filter press and centrifuge; use of wastewater for algal growth.	"Well to pump" analysis through RMEE method.	1 GJ of biodiesel
Stephenson et al. [26]	Comparison of cultivation design (ORP and tubular PBR) for microalgae growth; two-stage culturing approach: stage 1 with N sufficient supply and stage 2 with no N supply. Comparison of cell disruption methods (homogenization and cell hydrolysis). Anaerobic digestion of residual (lipids-extracted) biomass to generate electricity to be used on-site.	"Well to Wheels" analysis, including the emissions from microalgae biodiesel blended with conventional fossil-derived diesel. Foreground system: microalgae cultivation; harvesting; oil extraction and transesterification, background system: materials and energy used by the foreground system.	1 ton of biodiesel
Brentner et al. [72]	Comparison of microalgae cultivation design (i.e. ORP, annular PBR, tubular PBR and flat panel PBR); comparison of technologies for microalgae harvesting, lipids extraction and conversion to biodiesel (under economic allocation).	"Cradle to gate" analysis of five process steps, including: microalgae cultivation; harvesting; oil extraction and transesterification and by-products management. The analysis excludes transport infrastructure, labour, inputs and non-reactor capital equipment.	10 GJ of biodiesel
Collet et al. [125]	Assessment of the effects of an increased microalgae biomass productivity, biomass concentration and use of renewable source of electricity on climate change from microalgae derived biodiesel.	Attributional LCA of microalgae system including: biomass production; conversion to biodiesel (wet extraction); biodiesel combustion; construction/dismantling and disposal of culture infrastructure.	1 MJ of biodiesel
Quinn, et 2014 [74]	Assessment of the effects of an increased microalgae biomass productivity, extraction technologies (hexane vs. supercritical CO2) and integration of AD unit (allowing to nutrients recycling and CHP unit for on-site energy supply) on net energy ratio and life cycle GHG emissions.	"Well to pump" analysis of the energy and GHG emissions through GREET model. System includes: microalgae production; dewatering; lipids extraction and end-use of lipids-extracted biomass.	1 MJ of biodiesel
Woertz et al. [14]	Calculation of life cycle GHG emissions of microalgae biodiesel. Culturing of microalgae in ORP system by means of wastewater.	"Well to Wheels" analysis, including: microalgae cultivation; primary products transport; oil refining; fuel transportation to distribution terminal station and fuel combustion.	1 MJ LHV of biodiesel
Yuan et al. [67]	Development of mass balance model focusing on nutrients, carbon and energy flows through a microalgae biodiesel system with alternative technology options (four combinations of harvesting and dewatering options). Comparison of two fertilization conditions for microalgae growth, i.e. N sufficient supply and N starvation supply (i.e. based on the approximate molecular composition of microalgal biomass)	"Cradle to gate" analysis, including: algae cultivation; harvesting and dewatering; drying; oil extraction and utilization of residual biomass within the same facility. Next, the extracted oil is transported to a nearby biorefinery for biodiesel production. The analysis excludes: equipment; infrastructure construction, repair and maintenance; waste management.	1 MJ of biodiesel

Table A.2. Main features of the projected microalgae growing systems and site location considered by the different LCA studies under review.

Reference	Cultivation system design	Facility area (ha)	Site location
Lardon et al. [24]	ORP: system of 100 m length, 10 m width, 0.30 m depth. Operating regime: N-normal growth conditions.	100	Mediterranean location
Lardon et al. [24]	ORP: system of 100 m length, 10 m width, 0.30 m depth. Operating regime: N-low growth conditions.	100	Mediterranean location
Batan et al. [73]	PBR: system of 36 m length and 0.12 mm tick polyethylene bags supported in a thermal bath. The reactor bags are subdivide into three reactor sets, namely: incubation reactors to provide microalgae inoculums under N rich medium; reactor set for microalgae linear growth under nutrients rich conditions and reactor set for microalgae stationary growth under N-low conditions.	315	Colorado, US
Sander et al. [71]	ORP: system of 1.15 m length, 0.18 m depth. Operating regime: N-normal growth conditions (nutrients are supplied by wastewater).	unspecified	unspecified
Stephenson et al. [26]	ORP: system designed using two different ORP units, i.e: 1) ORP stage 1 of 150 m length, 10 m width, 0.30 m depth. Operating regime: N-normal growth conditions; 2) ORP stage 2 of 190 m length, 20 m width, 0.30 m depth. Operating regime: N-low growth conditions	1.21	United Kingdom
Brentner et al. [72]	ORP system of 77 m length, 14m width, 0.20 m depth. of 100 m length, 10m width, 30 cm depth. Operating regime: N-normal growth conditions	1.3	Phoenix, US
Brentner et al. [72]	Annular PBR cylinder of 2 m height, 0.5 m width, radius of 0.2 m. Operating regime: N-normal growth conditions	1	Phoenix, US
Brentner et al. [72]	Tubular PBR of 2.5 m height, 0.75 m width, 2 m depth. Operating regime: N-normal growth conditions	0.1	Phoenix, US
Brentner et al. [72]	Flat panel PBR of 2.5 m length, 1.5 m height, 1.5 m width. Operating regime: N-normal growth conditions	1.4	Phoenix, US
Collet et al. [125]	Pond for inoculums conservation and culturing ORP of 310 m length and 30 m width, 45 cm depth, 30 cm water depth. ORP are excavated and made by polypropylene liner; covered with a liner of polyethylene; ponds are covered by a removable greenhouse (made by flexible polyethylene film fixed to a wooden frame) to maintain a favourable temperature for microalgae growth while reducing water loss due to evaporation. Operating regime: N-low growth conditions	80	Mediterranean site (shrub land)
Quinn, et 2014 [74]	Three stages bioreactor system, including: 1) low volume closed bioreactor (under N-normal growth conditions) for supplying inoculum for large-scale facility; 2) high volume ORP facility; 3) section of the ORP dedicated to microalgae lipids accumulation (N-low growth conditions). Down-flow U-Tube configuration to minimize the energy to move the culture from bioreactor to processing facilities. Unspecified dimension	unspecified	unspecified
Woertz et al. [14]	High rate ORP system of 0.30 m depth.	4	Southern California, US
Yuan et al. [67]	ORP system of 0.30 m depth. Operating regime: N-normal growth conditions	unspecified	Southern New Mexico

Table A.3. Inputs of nutrients (N and P), CO_2 and water that are required for the cultivation of different microalgae strains, as documented from reviewed LCA studies. Results are reported to the functional unit of 1 kg of dry wt. algae.

Reference	Microalgae strain	Cultivation unit	N growth conditions	Nitrogen		Phosphorus		CO_2		Water	
				g N/kg	source	g P/kg	source	kg/kg	Flue gas source	l/kg	source
Lardon et al. [24]	*Chlorella v.*	ORP	N-normal	46.03	calcium nitrate	7.4	superphosphate	1.76	power plant	4	freshwater
Lardon et al. [24]	*Chlorella v.*	ORP	N-low	10.94	calcium nitrate	1.8	superphosphate	2.10	power plant	4	freshwater
Batan et al. [73]	*Nannochloropsis salina*	PBR	N-normal+N-low	147	unspecified	20	unspecified	unspecified	CO_2 enriched air	unspecified	unspecified
Sander et al. [71]	*Mixed strains*	ORP	unspecified	/	/	/	/	2.02	boiler, furnace or power plant	unspecified	WW (supplying N,P)
Stephenson et al. [26]	*Chlorella v.*	ORP	N-normal+N-low	65.89	ammonium nitrate	13.24	triple superphosphate	1.88	power plant	1.3	freshwater
Brentner et al. [72]	*Scenedesmus d.*	ORP/PBR	N-normal	60.26	ammonium nitrate	13.32	calcium phosphate	1.79	flue gas from power or ammonia plant	unspecified	freshwater
Collet et al. [125]	*Nannochloropsis occulata*	ORP	N-low	41.3	ammonium nitrate	8.9	diammonium phosphate	2.02	fume gas	unspecified	seawater
Quinn et al. [74]	*Nannochloropsis salina*	PBR+ORP	N-normal/N-low stages	18 [(a)]	Urea	27 [(a)]	diammonium phosphate	unspecified	flue gas power plant	unspecified	unspecified
Quinn et al. [74]	*Nannochloropsis salina*	PBR+ORP	N-normal/N-low stages	53 [(b)]	Urea	13.14 [(b)]	diammonium phosphate	unspecified	flue gas power plant	unspecified	unspecified
Woertz et al. [14]	*Mixed strains*	ORP	N-low	/	/	/	/	unspecified	flue gas power plant	unspecified	WW (supplying N,P)
Yuan et al [67]	*Scenedesmus d.*	ORP	N-normal	52.5	Urea	13.24	monopotassium phosphate	1.83	flue gas power plant	239	groundwater (light to medium salinity)
Yuan et al. [67]	*Scenedesmus d.*	ORP	N-low	17.5	Urea	13.85	monopotassium phosphate	1.83	flue gas power plant	373	groundwater (light to medium salinity)

[(a)] Referred to the system integrating the algae cultivation and the AD of residual LEA biomass; [(b)] Referred to the system excluding the AD of residual LEA biomass.

Table A.4. Biomass productivity, chemical composition (in terms of lipids, carbohydrates, proteins and ash) and lower heating value (LHV) of selected microalgae strains under different culturing systems (ORP/PBR designs and N supplies), as assumed by the different LCA studies under review.

Reference	Microalgae strain	Cultivation unit	N growth conditions	Biomass productivity ($g/m^2/day$)	Lipids (% dry wt.)	Carbohydrates (% dry wt.)	Proteins (% dry wt.)	Ash/others (% dry wt.)	LHV (MJ/kg)
Lardon et al. [24]	*Chlorella v.*	ORP	N-normal	24.75	17.5	49.5	28.2	4.8	17.5
Lardon et al. [24]	*Chlorella v.*	ORP	N-low	19.25	38.5	52.9	6.7	1.9	22.7
Batan et al., [73]	*Nannochloropsis salina*	PBR	N-normal+N-low	25	50	unspecified	unspecified	unspecified	unspecified
Sander et al. [71]	*Mixed strains*	ORP	unspecified	5	30	31	37.5	1.5	unspecified
Stephenson et al. [26]	*Chlorella v.*	ORP	N-normal+N-low	11	40	unspecified	unspecified	unspecified	unspecified
Brentner et al. [72]	*Scenedesmus d.*	ORP	N-normal	48	unspecified	unspecified	unspecified	unspecified	unspecified
Brentner et al. [72]	*Scenedesmus d.*	annular PBR	N-normal	96	unspecified	unspecified	unspecified	unspecified	unspecified
Brentner et al. [72]	*Scenedesmus d.*	tubular PBR	N-normal	646	unspecified	unspecified	unspecified	unspecified	unspecified
Brentner et al. [72]	*Scenedesmus d.*	flat panel PBR	N-normal	68	unspecified	unspecified	unspecified	unspecified	unspecified
Collet et al. **[125]**	*Nannochloropsis occulata*	ORP	N-low	20	45.7	16	22.3	15.9(b)	23
Quinn et al. [74]	*Nannochloropsis salina*	PBR+ORP	N-normal/N-low stages	25	50	unspecified	unspecified	unspecified	unspecified
Woertz et al. [14]	*Mixed strains*	ORP	N-low	22	30	37.5	37.5	/	unspecified
Yuan et al [67]	*Scenedesmus d.*	ORP	N-normal	25	25	35	32	8	19.1
Yuan et al. [67]	*Scenedesmus d.*	ORP	N-low	16	40	41	11	8	22.4

Table A.5. Overview of the alternative technology options at the different steps of the process chain, such as the harvesting-dewatering; drying; lipids extraction and their conversion to biodiesel, from previous LCA studies.

Process step	Reference Lardon et al. [24]	Batan et al., [73]	Sander et al. [71]	Stephenson et al. [26]	Brentner et al. [72]	Collet et al. [125]	Quinn et al. [74]	Woertz et al. [14]	Yuan et al. [67]
Harvesting-dewatering	Flocculation-filtration	Centrifugation-filtration	• Centrifugation; • Chamber filter press	Flocculation-centrifugation	• Centrifugation; • Chamber filter press; • Flocculation	Flocculation-sedimentation-centrifugation	Bioflocculation-DAF-centrifugation;	Bioflocculation-tickening by gravity	Bioflocculation-DAF-centrifugation
Efficiency (%)	90	unspecified	unspecified	unspecified	unspecified	95	95		90
Drying	Dryer (for dry extraction route)	/	Solar drying	/	Dryer	Dryer	Dryer	Solar dryer-flash dryer	/
Efficiency (%)	unspecified	unspecified	unspecified	unspecified	91	unspecified	90	unspecified	95
Extraction	• Hexane extraction (dry route) • Hexane extraction (wet route)	Hexane+ethanol mixture(a)	Hexane extraction	Cells disruption-hexane extraction	• Hexane extraction (dry route); • $ScCO_2$ (wet route); • ultrasonic+direct esterification (dry route); • ScMethanol (wet route)	• Hexane extraction (dry route)	• Pressure homogeneization; hexane extraction (dry route) • Pressure homogeneization; $ScCO_2$ (dry route)		Cells disruption-hexane extraction (wet route)
Efficiency (%)	70	90	/	/	95	/	90	/	73.6
Conversion	Transeste-rification	Transesterifi-cation	Transesterifi-cation[(b)]	Transesterifi-cation[(c)]	Transesteri-fication	Transesterifi-cation	/	Transesterifi-cation	Transesterifi-cation
Efficiency (%)	unspecified			99	98				

(a): mixture of hexane to ethanol at a ratio of 9:1 and a solvent to oil ratio of 22:1

(b): methanol to oil ratio of 6:1

(c): methanol and chloroform mixture

Table A.6. Summary of the main objectives, systems boundaries and functional unit (FU) of previous LCA studies on microalgae pyrolysis and HTL scenarios.

Reference	Objectives of the LCA study	System boundaries	FU
Handler et al. [25]	Calculation of the life cycle (fossil) energy demand and GHG emissions of two microalgae biofuels scenarios, namely: Scenarios ORP_WW_dry route (with settling or DAF): culturing of mixed species in ORP system by means of (primarily treated) WW effluent. Fast pyrolysis of (dried) microalgae to produce "rapid thermal processing" (RTP) green fuel. Upgrading of RTP fuel to hydrocarbon biofuel (similar to petroleum gasoline) by catalytic hydroprocessing; Scenarios ORP_N-Normal_dry route (with settling or DAF): culturing of selected strain (*Nannochloropsis sp.*) in ORP system by means of brackish/saline water, with inputs of fertilizers and CO2. Fast pyrolysis of (dried) microalgae to produce Rapid Thermal Processing (RTP) green fuel. Upgrading of RTP fuel to hydrocarbon biofuel (similar to petroleum gasoline) by catalytic hydroprocessing;	"Well-to-wheels" analysis from microalgae cultivation to biofuel production. The model includes input parameters for cultivation, harvesting-dewatering, drying, bio-oil recovery through pyrolysis, bio-oil stabilization, bio-oil hydroprocessing and co-products use.	1 MJ of biofuel
Bennion et al. [28]	Calculation of the life cycle (fossil) energy demand and GHG emissions of two microalgae scenarios, each considering results of an existing laboratory-scaled system and industrial-scaled projected system, as following: Scenario ORP_N-Normal_dry route_exp: pyrolysis pathway, including a small scale modelled system based on the results of laboratory experiments, under optimal conditions; Scenario ORP_N-Normal_dry route_ind: pyrolysis pathway, including an industrial scale modelled system based on information extrapolated from literature/field data, while assuming a given rate of improvement in terms of biomass yields and energy efficiencies; Scenario ORP_N-Normal_wet route_exp: HTL pathway, including a small scale modelled system based on the results from laboratory experiments, under optimal conditions; Scenario ORP_N-Normal_wet route_exp: HTL pathway, including an industrial scale modelled system based on information extrapolated from literature/field data, while assuming a given rate of improvement in terms of biomass yields and energy efficiencies.	"Well-to-pump" model including: growth, dewatering, bio-oil recovery through pyrolysis or HTL, bio-oil stabilization, bio-oil conversion to renewable diesel, transport and distribution to consumers pump.	1 MJ of biofuel

Table A.7. Inputs of nutrients (N and P), CO_2 and water that are required for the cultivation of different microalgae strains, from previous LCA studies on pyrolysis or HTL scenarios. Results are referred to the production of 1 kg of dry wt. algae.

Reference	Microalgae strain	Cultivation unit	N growth conditions	Nitrogen		Phosphorus		CO_2		Water	
				g N/kg	source	g P/kg	source	kg/kg	Flue gas source	l/kg	source
Handler et al. [25] - ORP_WW_dry route (with settling or DAF)	Mixed strains	ORP	unspecified	unspecified	WW	unspecified	WW	unspecified	WW[(a)]	unspecified	WW
Handler et al. [25] - ORP_N-Normal_dry route (with settling or DAF)	*Nannochloropsis sp.*	ORP	unspecified	unspecified	unspecified fertilizer	unspecified	unspecified	unspecified	flue gas	unspecified	brackish or saline water
Bennion et al. [28] scenarios ORP_N-Normal_dry route/wet route_exp	*Scenedesmus d.*	ORP	unspecified	920	BG-11[(b)]	920	BG-11[(b)]	/	atmospheric CO_2	unspecified	unspecified
Bennion et al. [28] ORP_N-Normal_dry route/wet route_ind	*Scenedesmus d.*	ORP	unspecified	88.6[(c)]	Urea	3.4[(c)]	Diammonium phosphate	/	atmospheric CO_2	unspecified	unspecified

[(a)] Carbon sources from wastewater effluent, including: carbon compounds (remaining after primary treatment) and dissolved CO2;

[(b)] Growth medium that was supplied to the lab-scale cultivation system;

[(c)] Calculated from given data of urea and di-ammonium phosphate supplied to the system [28];

Table A.8. Overview of the biomass productivity, chemical composition and lower heating value (LHV) of selected microalgae strains under different growth systems, from LCA scenarios investigated in previous works.

Reference	Microalgae strain	Cultivation unit	N growth conditions	Biomass productivity ($g/m^2/day$)	Lipids (% dry wt.)	Carbohydrates (% dry wt.)	Proteins (% dry wt.)	Ash/others (% dry wt.)	LHV (MJ/kg dry wt.)
Handler et al. [25] - ORP_WW_dry route (with settling or DAF)	Mixed strains	ORP	unspecified	12	10	unspecified	unspecified	unspecified	unspecified
Handler et al. [25] - scenarios ORP_N-Normal_dry route (with settling or DAF)	*Nannochloropsis sp.*	ORP	unspecified	25	25	unspecified	unspecified	unspecified	unspecified
Bennion et al. [28] - scenarios ORP_N-Normal_dry route/wet route_exp	*Scenedesmus d.*	ORP	unspecified	6.5	unspecified	unspecified	unspecified	unspecified	24
Bennion et al. [28]- scenarios ORP_N-Normal_dry route/wet route_ind	*Scenedesmus d.*	ORP	unspecified	13	unspecified	unspecified	unspecified	unspecified	unspecified

Table A.9. Overview of the technology options employed at the different steps of the thermochemical processing pathways of microalgae (i.e. harvesting-dewatering; drying; bio-oil recovery and its conversion to biofuel through pyrolysis or HTL scenarios), from previous LCA studies. The percent solids contained in the biomass stream are based on a dry weight basis.

Process step	Reference Handler et al. [25] (scenarios ORP_WW/ N-Normal _dry route (with settling or DAF)	Bennion et al. [28] (ORP_N-Normal_dry route _exp)	Bennion et al. [28] (ORP_N-Normal_dry route_ind)	Bennion et al. [28] (ORP_N-Normal_wet route _exp)	Bennion et al. [28] (ORP_N-Normal_wet route_ind)
Harvesting-dewatering	Flocculation; Settling or DAF (10% solids); Centrifugation (26% solids)	Membrane filtration (4% solids); Centrifugation (22% solids)	Bio-flocculation (1% solids); DAF (1.5% solids); Centrifugation (24% solids)	Membrane filtration (4% solids); Centrifugation (22% solids)	Bio-flocculation (1% solids); DAF (1.5% solids); Centrifugation (24% solids)
Efficiency (%)	unspecified	unspecified	unspecified	unspecified	unspecified
Drying	Thermal drying (90% solids)[(a)]	Lyophilization (90% solids)	Rotary drum drying (80% solids)	/	/
Efficiency (%)	unspecified	85%	89%	/	/
Conversion	(Whole cell) Pyrolysis (RTP green fuel); Stabilization; Catalytic Hydroprocessing	(Whole cell) Pyrolysis Stabilization; Hydroprocessing	(Whole cell) Pyrolysis; Stabilization; Hydroprocessing	HTL; Stabilization; Hydroprocessing	HTL; Stabilization; Hydroprocessing
Efficiency (%)	unspecified	unspecified	51	unspecified	55
Fuel product	Hydrocarbon biofuel (similar to petroleum gasoline)	Renewable diesel	Renewable diesel	Renewable diesel	Renewable diesel

[(a)] For the drying of microalgal biomass, the heat is supplied from natural gas (up to 58% solids concentration in biomass) and pyrolysis flue gases (up to 90% solids concentration in biomass).

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