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Environmental Sustainability of Microalgae-Based Production Systems: Roadmap and Challenges towards the Industrial Implementation

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Abstract

Microalgae and cyanobacteria are a precious source for the production of biofuels/bioenergy, biomaterials and valuable biochemicals from different production systems and biorefineries. Beyond photosynthetic CO_2 conversion, microalgal systems can involve the valorisation of waste streams and the implementation of green chemistry, industrial symbiosis, and circular bioeconomy approaches. However, their sustainability is uncertain, thus their large-scale application is hindered. The numerous life cycle assessments (LCAs) performed so far are mostly based on lab-scale, scaled-up or literature data, leading to qualitative and controversial results. This paper reviews primary data-based LCA studies on microalgal pilot to industrial scale plants. Fourteen studies satisfied the selection criteria, despite they used primary data almost exclusively for cultivation and harvesting. The outlined current status (methodology, inventory, energy consumption and environmental impacts) highlighted the lack of uniformity in the applied methods and in the presentation of results, as well as some lack of transparency. Nevertheless, the review concluded that electricity consumption and infrastructure are major hotspots. Therefore, the use of renewable energy for supplying the process and of sunlight for biomass photosynthesis must be preferred. The upstream processes produce large impacts. Thus, suitable reactor, geographic location, and harvesting method should be selected. Biofuels are not competitive in most cases, but some promising multi-product scenarios have been assessed as well. To improve the environmental profile of microalgal high value compounds (e.g., astaxanthin), co-products valorisation and enhanced compound productivity should be enhanced. More efforts on LCA of large-scale plants are required, especially looking at integrated biorefinery concepts, to take a crucial step towards the implementation of sustainable commercial systems.

Keywords: microalgae; pilot; Life Cycle Assessment; sustainable development; photobioreactor; raceway pond.

1. Introduction

Phytoplankton micro-organisms such as microalgae and cyanobacteria offer several benefits and have attracted the attention of many researchers for a multitude of applications in the production of numerous valuable products by moving away from fossil and mineral resources. In the framework of the carbon neutral transition, microalgal photosynthesis can be exploited to capture CO_2 from exhaust gas emissions. As renewable raw material, microalgae have emerged as feedstock for biorefining, defined as “the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)” (de Jong et al., 2012). Beyond hydrocarbon biofuels and other energy products, microalgae are a potential natural source of several bio-based valuable material products in the nutraceutical, cosmetic, pharmaceutical and food sectors, such as bioactive and green compounds. Numerous process routes have been developed to obtain multiple outputs from the production chain. Moreover, microalgae have been proposed for the treatment and valorization of waste effluents (Alazaiza et al., 2022; Premaratne et al., 2022), e.g., secondary wastewater or centrate from anaerobic digestion supernatant, by including integrated approaches in the perspective of circular bioeconomy.

However, the technology readiness level is not at commercial stage, due to concerns about the environmental impacts in the life cycle (Ubando et al., 2022), to the poor economic feasibility (Acién Fernández et al., 2019) (high costs for investment and cultivation, high energy consumption), and to the related technical challenges (Annevelink et al., 2022) (e.g., microalgae productivity). Other barriers to the technology advancement have been identified in the insufficient legal assistance and funding for research and innovation projects (Dębowski et al., 2022). In this framework, the sustainable production of microalgal bio-based products presents several challenges to achieve performances competitive with established technologies. In the recent years, many research efforts have been addressed to the assessment and the enhancement of the environmental and economic sustainability of microalgal systems, as demonstrated by the large number of scientific publications, including numerous original research articles and several review papers, devoted to these topics. However, the lack of sufficient data from large-scale (pilot and near-full scale) plants is a crucial obstacle that stands in the way of the TRL advancement and of the commercial implementation of environmental-friendly and cost-effective microalgal technologies (Dębowski et al., 2022).

Regarding the environmental dimension, a chronological overview on the scientific publications is depicted in Fig. 1. The chart reports the yearly number of scientific articles (since 1960) related to three searches in the *Scopus* database:

i. microalgae;
ii. microalgae and environmental impacts (life cycle assessment, LCA);
iii. microalgae, LCA, and large scale (pilot) plants.

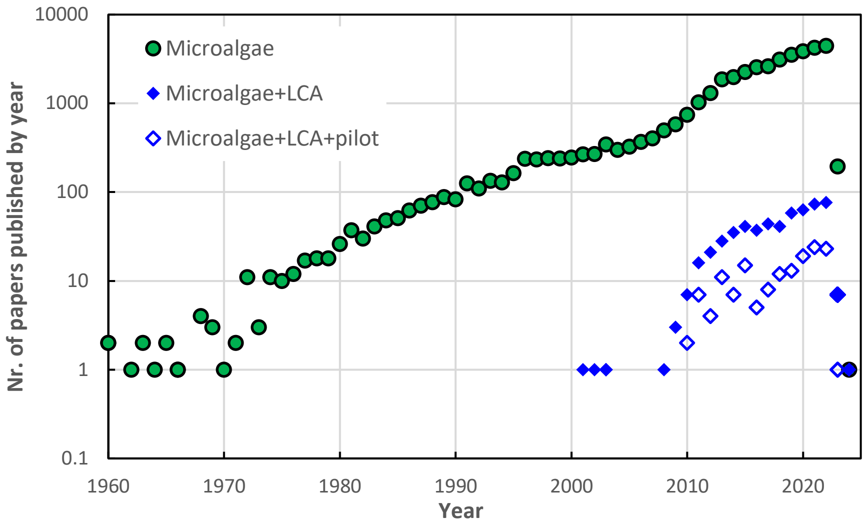


Fig. 1. Number of published papers by year since 1960 regarding microalgae, environmental aspects (life cycle assessment, LCA), and large scale (pilot) plants. Data retrieved from the *Scopus* database (www.scopus.com accessed on 20th December 2022) by different searches, as detailed in Section 2.

The number of publications related to microalgae has been continuously increasing up to the impressive value of 4446 in the year 2022, showing the great interest of researchers to this topic. The investigation of environmental issues has arisen in the last twenty years, with a significant increase since 2009, leading to 76 articles in 2022. Looking for LCA studies related to large scale microalgal plants, the search outcome shows a significant reduction in the number of scientific articles (23 in 2022). Overall, the total number of articles found with the above searches was 39,536, 554, 151, respectively. This shows that the literature is rich in studies regarding microalgae. However, the number of studies decreases of two orders of magnitude when including environmental aspects in the search, with studies related to large-scale plants being 27% of the total ones.

Note that the searches outcomes reported in Fig. 1 are raw results, i.e., they were not “filtered” in any way. Therefore, they may include some non-pertinent documents. However, Fig. 1 helps to illustrate the past, current, and future (projected) interest of the scientific community towards the general and specific topics that the present review focuses on.

Many reviews have been conducted so far on microalgae-based production systems, by including some treatment of sustainability aspects. However, only very few of them devoted some specific focus on large scale plants. On the contrary, most of them did not even distinguish large scale plants from laboratory apparatuses. In the following, reviews on LCA of microalgae-based production systems since 2019 are recalled. For the sake of brevity, review papers from the previous period are neglected.

He et al. (2023) conducted a review on carbon capture in microalgae cultivation and in biofuel production via hydrothermal liquefaction (HTL). Kim et al. (2022) reviewed studies on the technical development of microalgal biodiesel production. Nanda and Bharadvaja (2022) reviewed technical aspects of algal bioplastics and analysed their market. Ubando et al. (2022) reviewed LCA studies of microalgae biorefinery, thus addressing the central topic of the present paper. Morya et al. (2022) reviewed biotechnological and thermochemical pathways for bio-H_2 production from different organic feedstocks, including microalgae. Yadav et al. (2022) discussed economic and environmental (LCA) sustainability of microalgae biorefinery, with particular focus on value-added products. Goswami et al. (2022) addressed several technical aspects in biofuels production from microalgae, such as cultivation and harvesting phases, molecular or genetic approaches, artificial intelligence algorithms and internet of things-based sensors.

Liyanaarachchi et al. (2021) analysed engineering approaches for two-stage microalgae cultivation (biomass growth and target compounds accumulation occurring in two distinct steps) as a strategy of productivity enhancement. Merlo et al. (2021) reviewed studies on marine microalgal biofuels with a look at the sustainable development goals. Nagarajan et al. (2021) conducted a review on microalgal biohydrogen, discussing the process bottlenecks that hinder its commercialization. The review by Karpagam et al. (2021) focused on integrated bioprocessing methods for biodiesel and bioethanol production via transesterification and biochemical routes, as well as the potential use of spent microalgal biomass for near zero-waste residue applications. Devadas et al. (2021) conducted a review of studies on algae biopolymers in circular economy framework. Behera et al. (2021) focused on microalgae-derived biostimulants for plant growth.

Parsons et al. (2020) reviewed studies on oleaginous microalgae and yeast for making single cell oils, by evaluating economic and environmental impacts due to co-products. Rajesh Banu et al. (2020) reviewed articles on TEA, LCA and life cycle costing (LCC), and, by using literature data, they evaluated three different routes of algal integrated biorefinery for biofuels and valuable co-products. Gu et al. (2020) reviewed studies on hydrothermal liquefaction processes for the conversion of algal biomass into bio-oil and high value co-products.

Roy and Mohanty (2019) reviewed studies on microalgae harvesting techniques. Kumar and Singh (2019) conducted a review by analysing recent studies on microalgal biorefinery biodiesel production. De Souza et al. (2019) performed a bibliometric mapping from 2008 to 2018 and studied the association of microalgae with clean technologies. The review by Mishra et al. (2019) focused on the liquid and solid waste minimization in microalgal biorefinery. Wu and Chang (2019) described four biorefinery routes through LCA and TEA. Ubando et al. (2019) reviewed LCA papers on thermochemical processes for bioenergy products from microalgal (5 studies) or lignocellulosic (19 studies) biomass. Schade and Meier (2019) compared the environmental impacts of different production systems of microalgae for human nutrition, including a couple of studies with primary data at pilot scale. Bussa et al. (2019) investigated the potential of environmental improvement of using microalgal residues as a feedstock for polylactic acid in a multiple output system that produces lipopeptides. Koyande et al. (2019) focused on various products obtainable from microalgae bio-refinery.

Overall, the review papers published since 2019 mentioned above have not addressed sustainability dimensions assessed with input data from large-scale plants. This is due to the scarce presence (or even total absence in the past) of commercial facilities, but also to a lack of specific attention to the largest scale available plants. The same occurred in previous reviews of LCA (Carneiro et al., 2017; Ketzer et al., 2018; Lardon et al., 2009; Slade and Bauen, 2013). This represents a crucial lack of information hindering the technology advancement in view of a wide commercialization. Indeed, the LCA is sensitive to the scale of the system from which input data are drawn, and projections from lab scale experiments or engineering calculations may lead to misleading scaled-up results.

This paper proposes a novel review on environmental aspects of microalgae-based production systems by focusing on a selection of available studies regarding large scale (pilot and near-full scale) plants, in order to draw a picture that can be significant for realistic projections and for the technology scale-up. Following this rationale, we aim at (i) putting light on the current status and (ii) outlining a future prospect, thus providing insights useful for the scientific community devoted to the development of sustainable microalgae-based technologies projected towards future industrial implementations.

2. Research method and paper structure

The Scopus electronic database was used to search and collect scientific articles. Five searches were performed, as described in Table 1, without specifying any date range. The raw results in terms of yearly number of published papers are those reported above (Fig. 1).

The core of the present review paper was mainly based on documents from searches number 3 and 5 in Table 1, which were aimed at retrieving studies related to large scale (pilot) plants. A selection of articles was performed by checking the relevance and consistency of the articles with reference to the review aims. Therefore, only studies based on primary data from pilot plants producing valuable products, biofuels or bioenergy were selected (e.g., standalone microalgae plants for wastewater treatment were excluded). Moreover, full text availability and accessibility were considered, and in some cases other criteria related to the prestige of the study were included, such as the article type (e.g., conference proceedings were excluded). Some additional studies, pertinent with the aim of the present paper topic that met its eligibility criteria, were included by filtering papers from less restrictive searches (number 2 and 4 in Table 1). A bibliography was created by the *Mendeley desktop* software to organize the selected papers. Overall, the screening performed via full-text analysis filtered 14 relevant high-quality scientific articles for the review (excluding those used for the general parts of this paper).

Table 1. Searches performed in the *Scopus* database (www.scopus.com accessed on 20 December 2022) based on search words (with Boolean combinations) in article title, abstract or keywords. “SW” means search word, “Op” indicates the Boolean operator (either “AND” or “OR”), and quotation marks in a cell indicate that it has the same content as the corresponding one in the row above it.

Search #	**SW**	**Op**	**SW**	**Op**	**SW**	**Op**	**SW**	**Op**	**SW**	**Op**	**SW**	**Op**	**SW**
1	Microalgae	OR	Microalgal										
2	“	“	“	AND	Life cycle assessment	OR	LCA						
3	“	“	“	“	“	“	“	AND	Pilot	OR	Large scale	OR	Industrial

The present article is structured as follows. First, the general framework of microalgae biorefineries and process routes is described, including an overview on fundamentals of reactors for cultivation and of downstream phases after it (harvesting, extraction and purification, conversion), as well as of plant configurations and final products (Section 3). Then, the state-of-the art of environmental sustainability will be outlined regardless the plant scale (not only pilot) and the data source (not only primary data) (Section 4). The core of the work (review of the selected papers) will be represented by Sections 5 and 6, focusing respectively on the main features of the pilot plants and the relevant LCAs, with the discussion of the reviewed studies. Finally, the conclusions will be drawn, highlighting the main challenges and the key elements for future R&D activities (Section 7).

3. From microalgae cultivation to final products: an overview

Microalgae are an attractive feedstock because of the several advantages of their cultivation compared to other crops. For example, they can grow on non-arable lands in wastewater or seawater and are substantially more productive than land plants (Richmond and Hu, 2013). Moreover, microalgae raise interest because of their versatility in the obtainable products, such as biofuels and high-value compounds for several industrial sectors. Fig. 2 depicts a summary of the generic sequence of the main steps involved in the development and operation of a microalgal bioprocess system designed to obtain generic bioproducts. In the following, all steps are briefly described by distinguishing between upstream processes (preliminary steps, cultivation and harvesting) and downstream processes (cell disruption, extraction and purification, and residual biomass treatment).

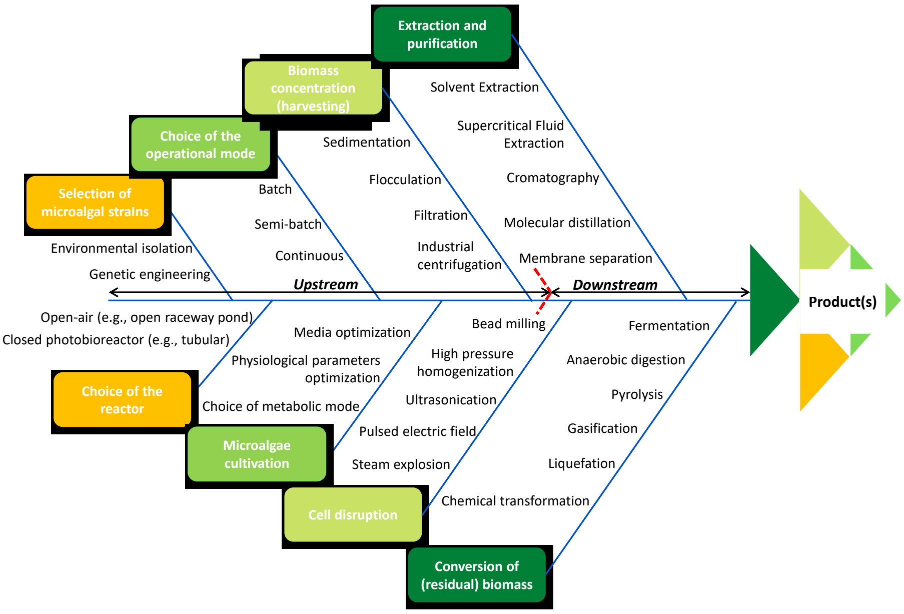


Fig. 2. Summary of the main steps involved in a bioprocess for bioproduct(s) from microalgae.

3.1. Upstream section

The first step of a microalgal bioprocess is the selection of the strain to cultivate. It is strictly connected to the desired application and to other criteria, such as its robustness and reliability under fluctuating outdoor cultivation conditions (Sen et al., 2022; Yun et al., 2019). *Scenedesmus*, *Chlorella* and *Nannochloropsis* have been identified as the most commercially important genera of microalgae (Laurens et al., 2017). In some cases, multiple strains are present as components of a consortium (Sen et al., 2022). This especially occurs when the main goal of the cultivation is wastewater remediation (Barreiro-Vescovo et al., 2020; Tejido-Nuñez et al., 2020), where it is impossible to maintain an axenic culture. In this application microalgae can be even coupled with other microorganisms, such as bacteria (Sánchez Zurano et al., 2020). As shown in Fig. 2, usually the selected strain is environmentally isolated, but there is the option of genetical improvement (Sen et al., 2022). However, there are at the moment only a few examples of transgenic microalgae cultivations at large scale (Zedler et al., 2016).

The three major constituents of microalgae are lipids, carbohydrates, and proteins, whose composition depends on microalgae species and culturing conditions. The content of various microalgae species is reported in Fig. 3, showing that most microalgae have high protein content. However, high content of lipids are observed in some cases (exceptional values are around 70% in Fig. 3, while Wu and Chang (2019) reported up to 53% and Koyande et al. (2019) reported up to 65.1%).

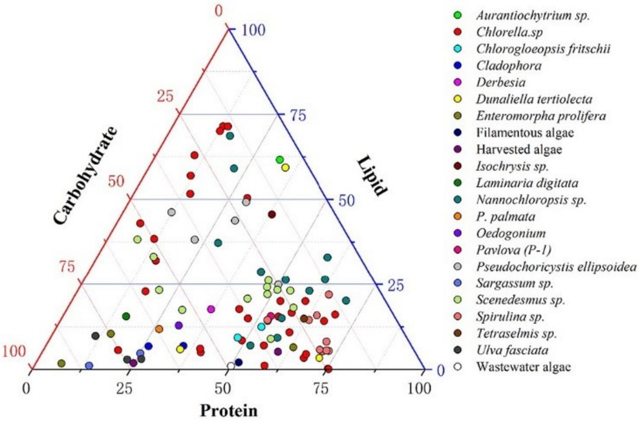


Fig. 3. Composition of various microalgae species. Reproduced from (He et al., 2023), with permission from Elsevier, 2022.

Bioreactors for microalgae cultivation can be divided into two main classes, i.e., open-air systems and closed systems (Xu et al., 2009), with the latter being mainly aimed at axenic single-species cultures (Fig. 2). Within the two classes of reactors, the most common typologies suitable for large-scale applications are open raceway ponds (ORPs) and tubular photobioreactors (PBRs). Nevertheless, several other types have been developed so far, including natural or artificial ponds and thin-layer cascade reactors among open-air systems (Fig. 4), and flat-plate, bubble column, airlift column and stirred tank reactors among closed systems (Fig. 5).

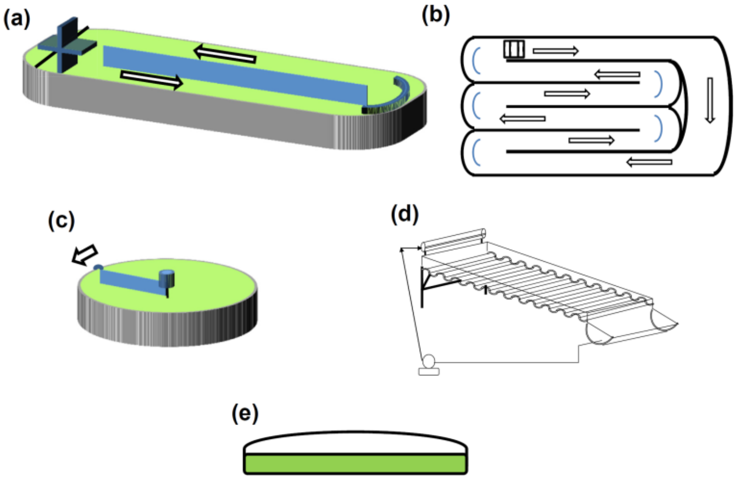


Fig. 4. Open systems for microalgae cultivation: (a) Raceway type, (b) multi-grid raceway, (c) circular type, (d) thin layer with undulating base, (e) covered pond. Reproduced from (Oncel, 2015), with permission from Elsevier, 2015.

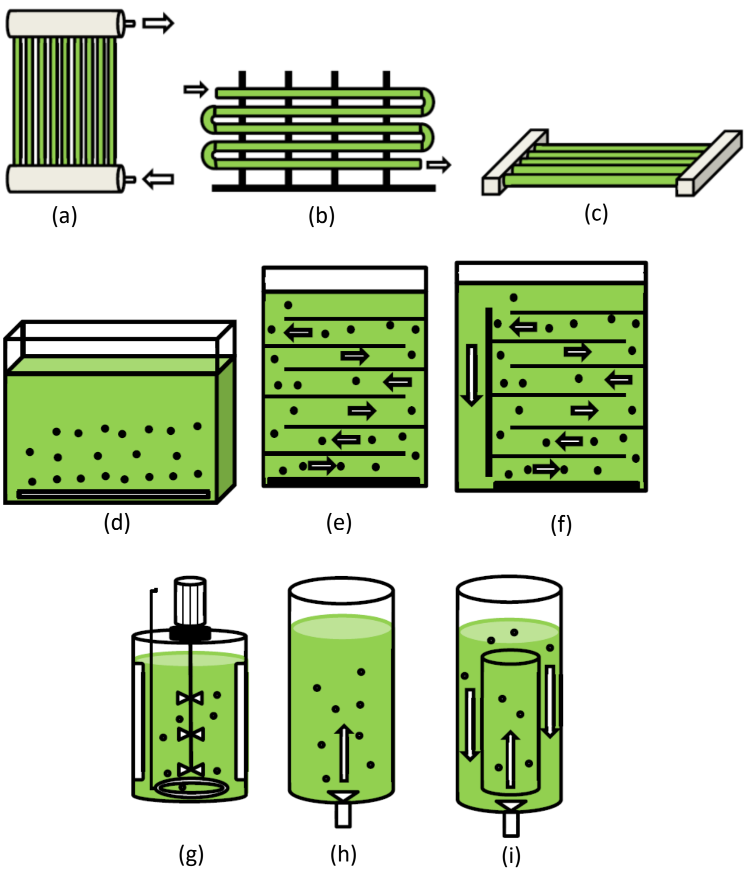


Fig. 5. Examples of closed systems for microalgae cultivation: (a) vertical tubular PBR, (b) tubular fence type with u-bends, (c) horizontal tubular PBR, (d) vertical flat panel, (e) panel with baffles, (f) airlift panel, (g) stirred tank, (h) bubble column, (i) airlift tank. Reproduced from (Oncel, 2015), with permission from Elsevier, 2015.

In the microalgal cultivation stage, one of the most important metrics is the biomass productivity, which is the biomass produced per unit time, either per unit volume of reactor or unit area occupied by the reactors. To increase the productivity, several parameters should be adjusted, such as the reactor design (for example, thin layer reactors are more productive than others), the reactor volume (for example, thin layer reactors are more productive than others), the culture medium, and the cultivation mode (batch, semi-continuous or continuous). Another critical factor is the choice of metabolic mode of the culture (photoautotrophic, mixotrophic or heterotrophic). Furthermore, intending to increase the productivity (of biomass or of a specific product), an optimization of the physiological parameters needs to be done. In the case of metabolite products, some stresses are often applied to the cultivation to stimulate the production of the biomolecule. Some of the employed stresses are high salinity and high light (Arena et al., 2021; Villanova et al., 2021), flashing light (Lima et al., 2022, 2021, 2020) and nitrogen deficiency (Solovchenko et al., 2008).

For the photoautotrophic cultivation, a crucial factor determining the biomass productivity is the annual irradiance on the geographic location in which the reactor is installed. A correlation between these two parameters is reported in Fig. 6, which gathers the areal productivity of several pilot scale reactors in which algae were grown without artificial lighting. The data collected from the literature indicate roughly a direct proportion between areal productivity and solar irradiation for open pond reactors. Thin layer reactors tend to be the most productive at pilot scale (in the set of analysed data the bigger reactor had a volume of 2500 L), while tubular stacked reactors are less productive.

Microalgae biomass productivity is often expressed per reactor volume. Typical values in large-scale cultivations are in the order of 0.01 to 1 g L^{-2} d^{-1} (Kim et al., 2022).

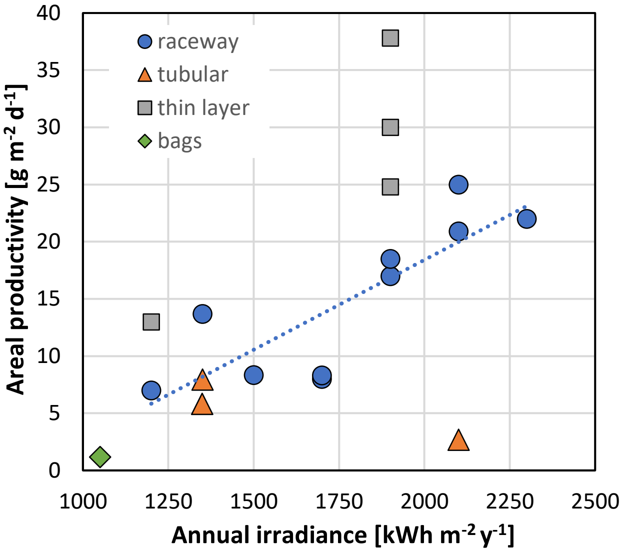


Fig. 6. Correlation between areal productivity of microalgal pilot plants and annual irradiance (sunlight photoautotrophic cultivation). Data on productivity regard 18 pilot cultivation systems documented in the literature (Amorim et al., 2021; Avila et al., 2022; Barreiro-Vescovo et al., 2020; Barros et al., 2019; Cavieres et al., 2021; Haines et al., 2022; Han et al., 2020; Iamtham and Sornchai, 2022; Long et al., 2022; Masojídek et al., 2022; Mohan et al., 2021; Montalvo et al., 2019; Morillas-España et al., 2021; Plouviez et al., 2019; Rentería-Mexía et al., 2022; Sánchez Zurano et al., 2020; Sung et al., 2021; Villaró et al., 2022). When seasonal data were available, the productivity value was calculated as annual average. Irradiance data come from Meteotest, Bern, Switzerland.

After the cultivation, microalgal harvesting is performed to separate or detach algae from its growth medium (Singh and Patidar, 2018). Commercial harvesting techniques produce a microalgae biomass concentration of 1-250 kg_{dw} m^{-3} (Gerardo et al., 2015). Dewatering of microalgae is a highly energy-consuming step, and it may be the major bottleneck in microalgae processing (Uduman et al., 2010). As mentioned in Fig. 2, several methods may be employed for microalgae harvesting, including sedimentation, flocculation, filtration and centrifugation (Gerardo et al., 2015). As pointed out by Gerardo et al. (2015), the choice of the harvesting method is not easy as it must take into consideration several process requirements, such as the separation mechanisms and the quality of the cells, as well as the operating and capital costs. For example, harvesting by centrifugation gives an optimal concentration range and quality of cells but has high capital and operational costs. The combination of methods is a strategy offering cost-effective solutions for harvesting (Singh and Patidar, 2018).

3.2. Downstream section and final products

After harvesting the biomass, downstream processes are devoted to extract valuable products. This may be achieved through many different process routes, depending on the desired final product(s) and the technology adopted in each step. Integrated approaches for muti-product extraction are certainly the most attractive from the economic and environmental sustainability. The downstream section often requires biomass pre-treatment before the extractive step. For example, drying is optional (that is why it is not indicated in Fig. 2) and is performed only in some cases (e.g., before products extraction from dry biomass via supercritical fluid) (Show et al., 2015). Some cell disruption methods are listed in Fig. 2, while a more exhaustive overview is provided in Fig. 7, including the main peculiarities of the different methods. The technique is to be chosen depending on the cell wall characteristics of the employed strain (Corrêa et al., 2020; Show et al., 2015). Mechanical and physical methods help the cell lysis through shear forces or energy transfer via waves or heat, while non-mechanical methods may be chemical or enzymatic. The cell disruption as a separate step is also optional, i.e., extraction methods can be used in combination with cellular disruption or directly applied over the whole cell (Corrêa et al., 2020).

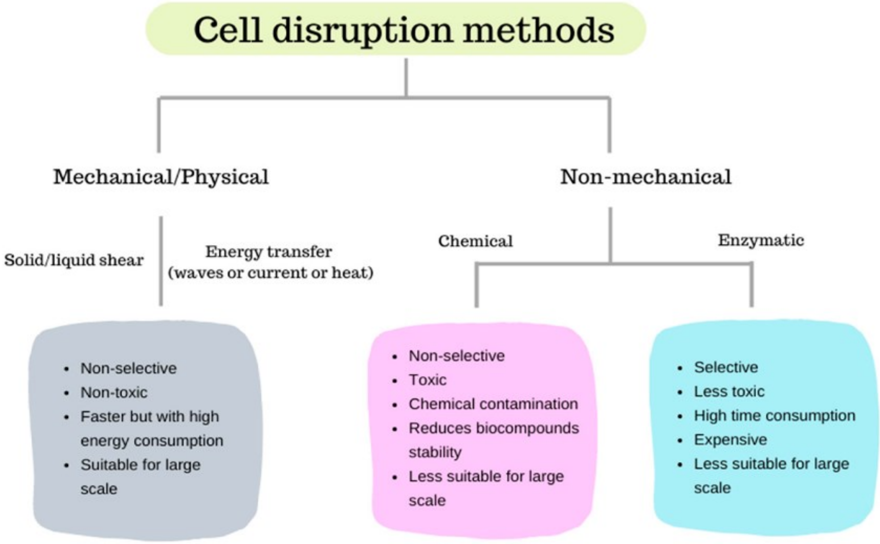


Fig. 7. Comparison of different cell-disruption methods, reproduced from (Corrêa et al., 2020).

Solvent extraction methods are the most common. Conventional organic solvents are employed in well-established techniques for the extraction of microalgae biocompounds, mostly lipids. However, conventional solvents are toxic and bring environmental and human health concerns. In contrast, alternative solvents are supposed to have lower environmental, safety and health impacts. They include ionic liquids, deep eutectic solvents, and supercritical fluids, among others (Corrêa et al., 2020). The choice of the solvent depends on the target compound, but non-conventional solvents have shown a significant potential for sustainable and scalable technologies with high efficiency and purity (Corrêa et al., 2020).

After extraction, a purification step is required in the case of target products represented by biomolecules. Separation methods for this purpose include electrophoresis, membrane separation, ultracentrifugation, among others (Corrêa et al., 2020). In biofuel production, an example of application of a purification process is oil refining to separate cellular debris, membrane lipids, and pigments from triglycerides (after extraction with solvent) (Branco-Vieira et al., 2020; Stephenson et al., 2010).

The downstream section may also include a conversion step to achieve the final product. For example, catalytic transesterification is a typical process applied to convert extracted lipids into fatty acid methyl esters (FAME), i.e., biodiesel. Overall, different transformation methods can be applied for biomass, either “raw” from harvesting/drying or residual from extraction. For biofuel / bioenergy production, there are several conversion methods and products (Mishra et al., 2019). Conversion techniques can be classified into four groups, as shown in Fig. 8. Microalgae are considered the best alternative feedstock for the production of 3rd generation biofuel. However, microalgal biofuel (e.g., biodiesel) production is not competitive yet due to various technical and economic constraints, and thus it is not commercially attractive (Rajesh Banu et al., 2020; Venkata Subhash et al., 2022).

The residual biomass can be considered a co-product to be directed to some final use, typically animal feeding (Beal et al., 2015). Otherwise, it may be employed in biofuel or bioenergy production through conversion techniques like anaerobic digestion or hydrothermal liquefaction (Karpagam et al., 2021; Venkata Subhash et al., 2022). The most common application is anaerobic digestion for biogas production (Hernández et al., 2014; Markou et al., 2022; Zabed et al., 2020), thus targeting to heat and / or electricity as final products, while hydrothermal liquefaction produces bio-oil.

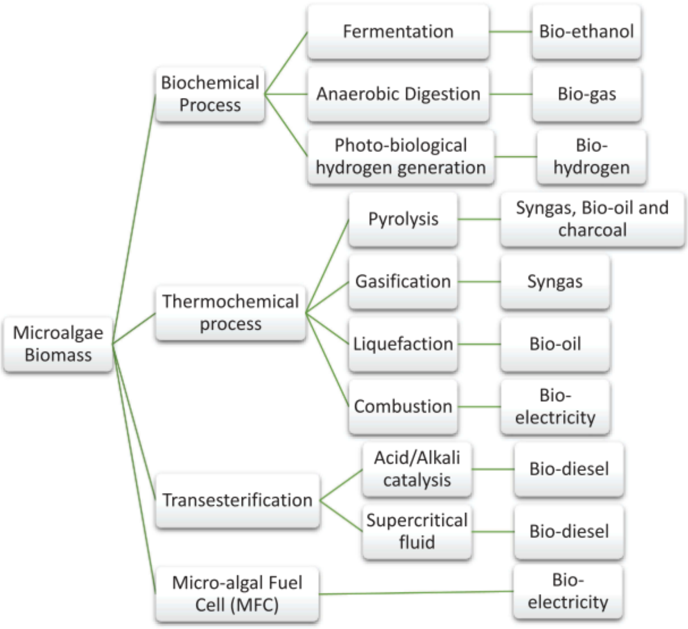
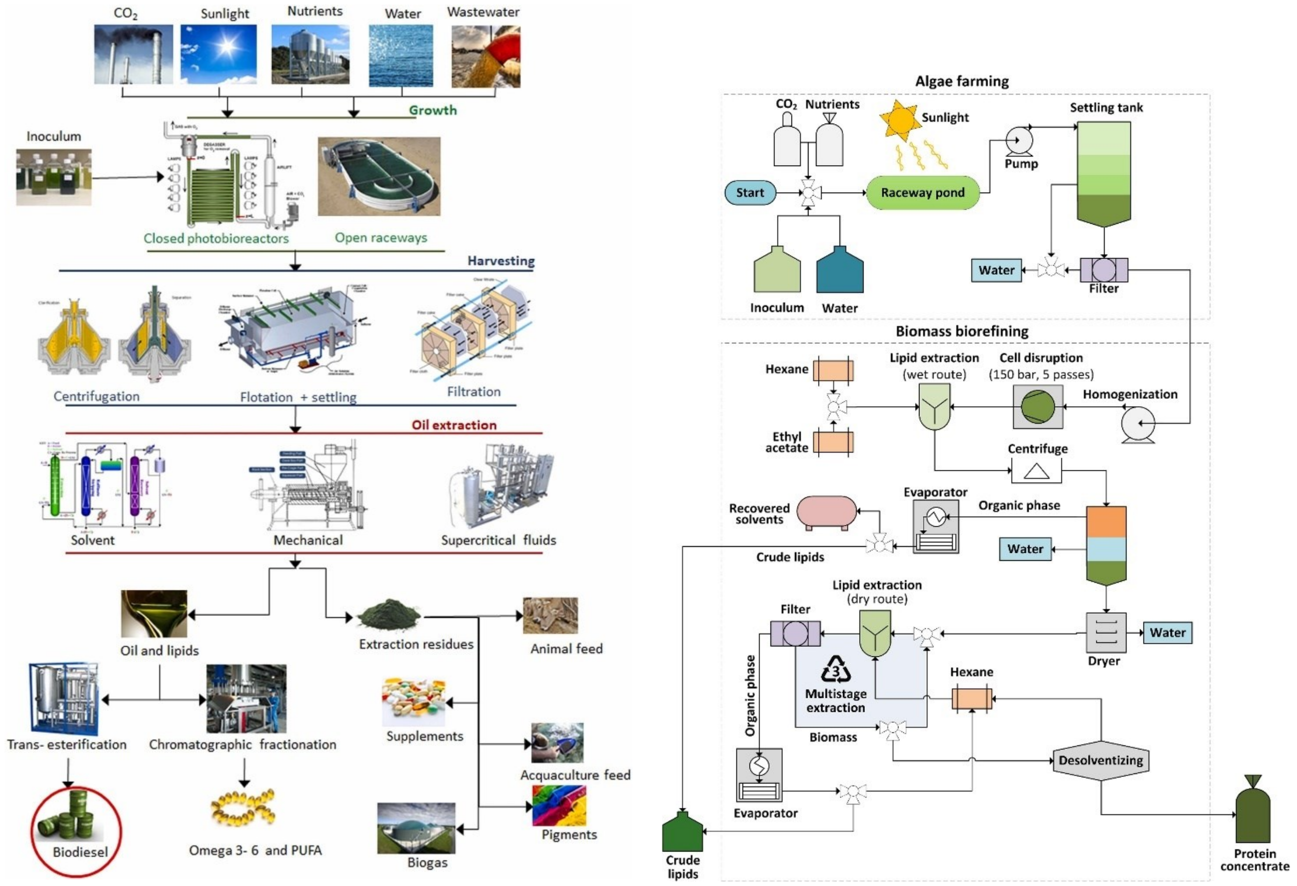


Fig. 8. Biofuel or bioenergy production from microalgae biomass. Reproduced from (Koyande et al., 2019).

Apart from fuel and energy, microalgae are a feedstock for products for food, feed, cosmetics, nutraceutical, and pharmaceutical applications because they produce and accumulate various macromolecules. Indeed, microalgae are a rich source of proteins, carbohydrates, pigments, PUFAs, peptides, and other important molecules. Thus, one of the main features of microalgae is the huge potential of extractable products, i.e., biofuels, biochemicals, and biomaterials (Laurens et al., 2017). A complete list of the count-less microalgae-derived products is arduous, but the most important ones can be mentioned: biofuels (Goswami et al., 2022; Mishra et al., 2019; Sen et al., 2022; Venkata Subhash et al., 2022), including biocrude (He et al., 2023), biodiesel (Katiyar et al., 2017; Kim et al., 2022), biogas (Zabed et al., 2020), and biohydrogen (Ahmed et al., 2022; Nagarajan et al., 2021); biopolymers (Devadas et al., 2021) and bioplastics (Nanda and Bharadvaja, 2022), biofertilizers (Braun and Colla, 2022) and biostimulants (Behera et al., 2021; Braun and Colla, 2022), high-added value products and bioactive compounds (Long et al., 2022; Olguín et al., 2022).

Microalgae are a source of natural pigments, including carotenoids (Di Lena et al., 2019; Zheng et al., 2022). This group of high-added value products from microalgae deserve a special attention, because they are characterized by an increasing trend in commercialization. Chlorophylls, phycocyanin, astaxanthin, and β-carotene are the microalgal pigments with the most significant global market, and the most studied strains for extracting them are *Chlorella vulgaris*, *Spirulina platensis*, *Haematococcus pluvialis*, and *Dunaliella salina* (Silva et al., 2020). Among carotenoid pigments, astaxanthin is a high-value compound derived from *Haematococcus pluvialis*. Natural astaxanthin is a “super anti-oxidant” for human applications (dietary supplements, cosmetics, and food and beverages) with a market value from 2500–7000 \$ kg^{-1} to about 15,000 \$ kg^{-1} depending on product purity (Shah et al., 2016).

Extraction of high-value compounds as co-products can improve viability of biofuel production (Goswami et al., 2022; Venkata Subhash et al., 2022) and can be devised through different routes (Rajesh Banu et al., 2020). The simultaneous exploitation of several fractions coming from the same biomass is the basis of the idea of microalgal biorefinery (Goswami et al., 2022; Gu et al., 2020; Karpagam et al., 2021; Koyande et al., 2019; Laurens et al., 2017; Mishra et al., 2019; Rajesh Banu et al., 2020; Venkata Mohan et al., 2020; Venkata Subhash et al., 2022; Yadav et al., 2022). With this expression, one refers to integrated processes that assure the valorisation of multiple fractions of the microalgal biomass. According to this strategy, several processes are integrated into an energy and non-energy multi-product route in order to decrease wastes and overall costs of the process minimize waste (Mishra et al., 2019). Fig. 9a illustrate the biorefinery concept by an example of a generic process scheme. Currently, there is not any complete biorefinery process operating at commercial scale able to exploit the microalgal biomass in all its possible components, i.e., raw biomass, lipids, polysaccharides, pigments, proteins, etc. (Barsanti and Gualtieri, 2018). Indeed, scaling up is inefficient due to some challenges at different stages of biorefinery (Yadav et al., 2022). However, the biorefinery concept for the production of biofuel and high-value products offers promising perspectives for economically and environmentally sustainable alternatives for bioeconomy industries.



(a) (b)

Fig. 9. Microalgal biorefinery: (a) example of a generic scheme; (b) *S. obliquus* biorefining for the production of crude lipids and protein concentrate. Reproduced from (a) Usai et al. (2023); (b) Amorim et al. (2021), with permission from Elsevier, copyright 2021.

Although scale factors may affect the competitiveness of microalgal biorefinery (Bose et al., 2022), it is undeniable that this integrated approach may be an attractive perspective as a source of clean technologies (de Souza et al., 2019; Rajesh Banu et al., 2020). Several biorefinery routes have been proposed so far (Amorim et al., 2021; Katiyar et al., 2021; Montalvo et al., 2019). Fig. 9b shows an example of biorefinery applied in a pilot scale cultivation of *Scenedesmus obliquus* with the co-production of crude lipids (biofuel precursor) and protein concentrate (animal feeding). The cultivation of *S. obliquus* occurred inside an open raceway pond in a culture medium called L4-m. The microalgal slurry was recovered through centrifugation and disrupted in a low-pressure homogenization device. After that, lipids were extracted via solid-liquid extraction in hexane and ethyl acetate. A second dry extraction of lipids was obtained with hexane from the dried microalgal paste. Then, the protein concentrate was obtained by evaporating residual hexane from the algal biomass recovered from the previous steps. The study achieved promising results, but integrated schemes deserve much more research efforts via the exploration of an array of options and the assessment of their sustainability.

4. Environmental sustainability of microalgae-based systems

Microalgae offer alternative routes for a wide spectrum of bio-based productive schemes. However, these are nascent technologies that need a robust and reliable assessment of sustainability aspects to define a baseline of the current status from which novel developments can start, leading to future industrial implementations.

As shown in Section 1, the scientific literature is prolific in the research related to environmental and economic characteristics of microalgae-based production systems. In regard to the environmental pillar of sustainability, Life Cycle Assessment (LCA) has been performed in numerous studies. LCA is a standardised methodology aimed at quantifying the environmental pressures (resource consumption, emissions, waste generation and associated environmental impacts) related to goods and services (products) by taking into account the full life cycle of the product. LCA has been used increasingly by industry to reduce the overall environmental burdens and to improve the competitiveness of the company’s products. LCA allows benchmarking of product system and can be used as decision making tool to improve product design and to orient technology investments and innovation. In the public sector, LCA equally makes use of life cycle thinking in stakeholder consultations and in policy implementation. LCA provides valuable information on environmental performance of goods and services, thus contributing to product policy to the analysis of the environmental performance of production and consumption patterns.

The LCA methodology is internationally standardised by the environmental management standards ISO 14040:2006 (*ISO 14040:2006 - Environmental management — Life cycle assessment — Principles and framework*, 2006) and 14044:2006 (*ISO 14044:2006 - Environmental management — Life cycle assessment — Requirements and guidelines*, 2006).

LCA is performed for a Functional Unit (FU), i.e., the quantified performance of a product system that provides a certain functionality, which is used as a reference unit. An LCA study consists of four phases: a) the goal and scope definition, b) the life cycle inventory (LCI) analysis, c) the life cycle impact assessment (LCIA), and d) the interpretation. The scope, including the system boundary and level of detail, of an LCA depends on the subject and the intended use of the study. The depth and the breadth of LCA can differ considerably depending on the goal of a particular LCA. The LCI is the collection and analysis of all elementary input/output flows (e.g., emissions to air and water, waste generation and resource consumption) which, in the most general case of system boundaries “from cradle to grave”, are associated with a product from the extraction of raw materials through production and use to final disposal, including recycling, reuse, and energy recovery. The LCIA is the estimation of indicators of the environmental pressures in terms of climate change, resource depletion, human health effects, etc. associated with the environmental interventions attributable to the life-cycle of a product. The interpretation phase serves to summarize and discuss the LCI and/or LCIA results, to draw conclusions, recommendations, and decision-making in accordance with the goal and scope definition. For details on theory and practice, see the pertinent literature, e.g., (Hauschild et al., 2018). Despite there are conceptual aspects still debated, i.e., without unanimous consensus (Schaubroeck et al., 2021), the LCA methodology is undoubtedly recognized as a powerful and worthwhile tool. It is the most adopted method for the sustainability evaluation of bio-based technologies (Escobar and Laibach, 2021).

4.1. Bibliometric mapping

In order to take a picture of the literature regarding microalgae and sustainability, a brief scientometric analysis was performed. Bibliometric data from search number 2 in Table 1, regarding microalgae and environmental (LCA) aspects, were extracted to create two distinct networks of co-occurrence of authors' keywords, reported in Fig. 10. Each circle represents a keyword with a minimum occurrence of 6-fold. Font size and circle size of keywords reflect the number of occurrences, while each colour identifies a cluster (or group) of related keywords. The network analysis based on the results reported in Fig. 10 reveals the research hotspots in the field of environmental sustainability of microalgae systems.

"Microalgae" and "LCA" keywords belong to the main cluster (including 9 items in total) and are related by a strong link. The third and fourth keywords with the highest occurrences are "biofuel" and "biodiesel", showing that most LCAs of microalgae systems have been conducted for process schemes involving biofuels as main products. It is further highlighted by the "biorefinery" keyword, which ranks seventh in terms of occurrences. As it may be expected, "sustainability" and "environmental impact" are keywords occurring many times. Interestingly, "TEA" is found with several occurrences in the search for microalgae and LCA, showing that environmental and economic dimensions are often mentioned together. Note that wastewater and anaerobic digestion play a major role, mainly framed within circular bioeconomy concepts and biofuel / bioenergy production. *Chlorella vulgaris* is the algal species by far most mentioned among the authors' keywords in both searches. "Cyanobacteria" is a quite recurring keyword as well, showing that other phytoplankton groups are associated to applications very similar compared to microalgae, despite they belong to a different domain.

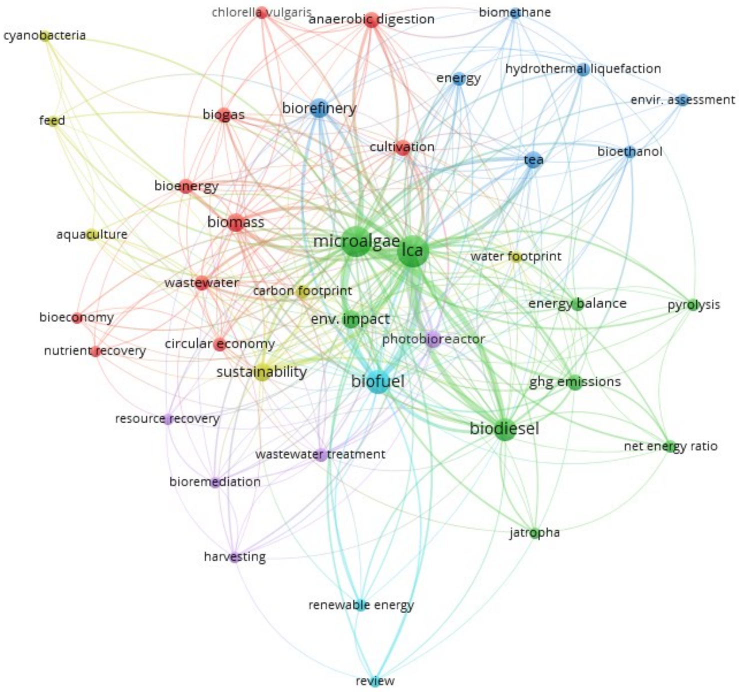


Fig. 10. Keyword co-occurrence network map related to microalgae and environmental assessment (LCA). Data retrieved from the Scopus database (search number 2 in Table 1) and elaborated by the VOSviewer 1.6.18 software. A minimum number of occurrences of a keyword equal to 6 was set. Out of 1180 keywords, 56 met this threshold. Equivalent keywords (e.g., life cycle assessment and LCA) were merged, resulting in a final number of keywords represented in the network equal to 40.

4.2. Qualitative meta-review

Recent advancements on the environmental performance of microalgae-based production systems are discussed in this section by an analysis of previous review papers. A summary of the main results and conclusions will be discussed, by providing a comprehensive framework from which our treatment focused on large scale plants can start.

By reviewing LCA and TEA studies, He et al. (2023) concluded that cultivation and HTL processes have negative CO_2 emission and reduced energy consumption (compared to other biomass conversion methods) in biofuel production. However, comparisons among results from different LCA studies were difficult due to the lack of standardization in the adopted methods. Estimated GHG emissions for algal-based biofuels spanned in a wide range from -75 to 534 $gCO_{2\text{-eq}}$ MJ^{-1}, thus making greatly uncertain the mean result. The highest CO_2 fixation rate (80-260 gCO_2 m^{-3} h^{-1}) was provided by *Chlorella vulgaris*. Recent studies showed that integrating wastewater treatment with algae cultivation has the potential to make the biodiesel production more sustainable. Values of net energy ratio (NER), defined as output energy over input energy, of HTL systems ranged broadly from ~0.34 to 1.25. The values of Energy Return on Investment (EROI, given by the energy produced over the energy required, including direct and indirect contributions) were significantly lower than 1 or approximatively 1 (the higher values obtained with HTL), thus requiring the investigation of novel processes with potential of enhanced competitiveness.

Microalgae drying and lipid extraction contributed to more than 70% of Global Warming Potential (GWP) in microalgal biodiesel production systems (Kim et al., 2022). Direct transesterification of microalgae into biodiesel without lipid extraction was proposed as an alternative option with improved efficiency. However, LCA studies of microalgal biodiesel production at supercritical conditions and non-catalytic transesterification are required.

In the production pathways for microalgal bioplastics, the most attractive approach was identified with a biorefinery that integrates multiple value-added products and wastewater remediation (Nanda and Bharadvaja, 2022). Cradle to gate GHG emissions for bioplastics were 0.4–1.3 ton CO_2 ton^{-1}, which are lower than those for petrochemical based plastics (1.8–3.55 ton CO_2 ton^{-1}). In the end-of-life phase, which is a key portion in the life cycle, mechanical recycling was widely identified as the most suitable disposal method for bioplastics with least carbon footprint (0.62 kg CO_2 kg^{-1} polylactic acid) compared to anaerobic digestion, landfilling, composting and incineration.

By reviewing LCA studies of microalgae biorefinery, Ubando et al. (2022) highlighted that the LCA methodology has been helpful in identifying the environmental bottlenecks, and that green technologies may lessen GHG emissions and enhance profit. Microalgae biorefinery was recognized to be still challenging across both environmental and economic dimensions. By considering a selection of eleven studies on microalgae biorefinery, the GWP was lower than 30 g$CO_{2\text{-eq}}$ MJ^{-1} in most cases, but it could be over 15,000 g$CO_{2\text{-eq}}$ MJ^{-1} in case of CO_2 intensive processes. However, a negative value was reported as well (carbon credit). NER values of 2.14-2.23 or 0.14-0.3 were reported for a co-digestion system and a process with HTL, hydrotreating, and hydrocracking, respectively. Note that the latter range was for the definition of NER as total energy input to total fuel higher heating value (Chen and Quinn, 2021). Among the weaknesses of LCA studies, the use of different functional units was clearly highlighted. This heterogeneity in the applied methods makes comparisons of LCI and LCIA results from different studies difficult. On the other hand, coupling TEA with LCA was prospected as a successful methodological strategy for a comprehensive assessment of sustainability.

A greater research attention and exhaustive LCA was recommended for microalgal bio-H_2 production by Morya et al. (2022). Compared to fossil fuels, bio-H_2 for electricity production could reduce GHG emissions. Moreover, bio-H_2 or methane production via reactive flash volatilization assisted from microalgae had lower emissions (7.56 kg $CO_{2\text{-eq}}$ kg^{-1} H_2 or 1.18 kg $CO_{2\text{-eq}}$ kg^{-1} CH_4) than steam reforming of methane (11.9 kg $CO_{2\text{-eq}}$ kg^{-1} H_2) or methane production (4.25 kg $CO_{2\text{-eq}}$ kg^{-1} CH_4). Data from another study regarded microalgae production performance. Across different scenarios, the estimated fossil EROI varied from 0.38 to 1.08, while life cycle GHG emissions were −46.2 to 48.9 g $CO_{2\text{-eq}}$ MJ^{-1} and water demand was of 20.8 to 38.8 L MJ^{-1}.

Yadav et al. (2022) emphasized LCA and TEA as important tools to promote the sustainable production of biofuels, bioactive and nutraceutical compounds, and green products. Lipid content enhancement and microalgae cultivation in wastewater with subsequent thermo-chemical processing by hydrothermal liquefaction (HTL) were highlighted for the sustainable production of biodiesel. Overall, the integrated algal biorefinery approach for the simultaneous production of biodiesel and value-added co-products was identified as a successful strategy for the deployment of feasible and sustainable process schemes. A favourable NER (higher or lower than 1, depending on the definition) was indicated as a crucial requirement for a cost-effective biorefinery. Unfavourable NER values for bioenergy production through HTL or pyrolysis were 1.23 or 2.27, while GHG emissions were of −11.4 or 210 g $CO_{2\text{-eq}}$ MJ^{-1}.

Goswami et al. (2022) found that ~90 % of the LCA and TEA studies were conducted by focusing only on biofuels or single biorefinery processes, while novel scenarios with co-products and assessments based on large-scale experimental data require more attention. Artificial intelligence or sensors based on internet of things along with modelling tools can be effective in microalgae strain selection and medium optimization. Integrating wastewater treatment, CO_2 mitigation, co-product formation (biofertilizers and bioplastics), molecular or genetic approaches, is strategic for reducing environmental impacts and economic costs. LCA results included GHG emissions of 0.112 kg $CO_{2\text{-eq}}$ MJ^{-1} EtOH and 0.039 kg $CO_{2\text{-eq}}$ MJ^{-1} FAME, respectively, in the production of bioethanol and biodiesel. Lipid extraction after mild hydrothermal treatment was the best method for biodiesel production, leading to good performance in terms of NER and of several environmental impacts. The highest NER of 18.8 was reported for a wastewater-biocatalytic transesterification scenario. In the production of biogas, anaerobic digestion with hydrothermal pre-treatment vs. anaerobic digestion with solar-driven hydrothermal pre-treatment resulted into NER (as energy input over energy output) of 0.54 vs. 0.69, and GHG emissions of –129.4 vs. –169.13 g $CO_{2\text{-eq}}$ kWh^{-1} biogas.

Two-stage microalgae cultivation can increase the productivity of target metabolites, but may require higher capital and operating costs (Liyanaarachchi et al., 2021). NER and GWP are the two most evaluated metrics in LCA of microalgal biofuels, with a general consensus in the literature in showing better performance of raceway ponds over PBRs. A factor of 5 in the NER characterize the lower performance of microalgal biofuel compared to petroleum-based diesel. In contrast, the GWP of microalgae systems is significantly lower. The use of renewable energy sources, culture media recycling, nutrient recovery from wastewater, enhancing biochemical composition, improving calorific value of products and cost-effective downstream processing can improve NER and GWP of microalgae-based biofuels. LCA studies on two-stage cultivation have reported contradictory results for these indicators. Moreover, other impact categories should be considered in future studies.

Merlo et al. (2021) concluded that the large-scale production of marine microalgae biofuels is not yet economically feasible. They showed that, despite the obtainment of contradictory results, many LCA studies evaluated that microalgal biofuels reduce GHG emissions. Nutrients recycling is essential for improving GHG and energy balances, but there is an urgent necessity to assess large scale facilities, thus generating accurate and reliable results overcoming limitations and assumptions of current LCA studies. Harvesting and dewatering were identified as the most energy intensive and the most expensive (contribution of 20–30%) phases. NER values of 1.08 or 1.46 were reported for production systems based on PBRs or raceway ponds, respectively. Land and water requirement were $6.18E^{-4}$-$7.30E^{-4}$ ha GJ^{-1} y^{-1} and 98 L GJ^{-1} y^{-1}, respectively, while net GHG emissions were –0.075 $tCO_{2\text{-eq}}$ GJ^{-1} y^{-1}.

Nagarajan et al. (2021) claimed that TEAs and LCAs on biohydrogen production from microalgae are very few. Results from LCA studies showed an NER of 6, but also a cumulative energy demand (CED) higher than common technologies of hydrogen production. With a production of 0.0114 kg H_2 kg^{-1} biomass, the energy consumption and CO_2 emissions were of 1538 MJ MJ^{-1} H_2 and 114,640 gCO_2 MJ^{-1} H_2, respectively.

The review by Karpagam et al. (2021) indicated the opportunity of integrating bio-energy production with waste remediation for sustainable applications of microalgal routes, reducing emissions to 4.2 kg $CO_{2\text{-eq}}$ kg^{-1}.

In the production of biopolymers from microalgae, the sustainability is affected by uncertainties on the scale-up (Devadas et al., 2021). However, integrated approaches involving the use of waste can lead to GHG emission credits.

In the production of biostimulants, LCA identified cultivation and extraction as the major steps contributing to environmental and ecological impacts. For example, inoculation and culture contributed by 73% to 90% across the impact categories. The use of fuel-derived electricity from the grid was responsible of 51% of total emissions.

Among the co-products of single cell oils obtainable from algae and yeast, the protein fraction was identified as crucial to determining minimum oil selling price and environmental impacts (Parsons et al., 2020). Two different biofuels pathways were characterized by emissions of 162 vs 5.3 kg $CO_{2\text{-eq}}$ GGE^{-1}. By comparing the production of animal feed with nutrient recovery along with energy generation, the revenue was almost doubled in the first option. In contrast, GWP was lower in the second option, despite it was more uncertain. From the methodological point of view, it was stressed that in assessments with co-products (i) the choice of the functional unit must reflect the aims and objectives of the study, and (ii) allocation must be clearly discussed.

Commercialization of microalgal biorefinery was not economically feasible (Rajesh Banu et al., 2020). A broad range of variability was reported for the NER (~0.1 to 3). Comparing several LCA studies, the adopted methods and main results were quite heterogeneous.

By analysing LCA studies, Gu et al. (2020) highlighted the importance of using flue gas and recycle nutrients (e.g., by anaerobic digestion) to enhance NER and mitigate GHG emissions. Compared to other technologies for bio-oil production, hydrothermal liquefaction (HTL) showed a general trend of better environmental performance. A lack in the literature was claimed in the application of LCA for the two-stage sequential HTL process, which has the potential to produce valuable co-products.

In the harvesting phase, high energy demand and high GHG emissions were identified (Roy and Mohanty, 2019). Energy consumption involved in harvesting from PBR systems ranged below 0.5 kWh kg^{-1}, outperforming open systems (4.5 kWh kg^{-1}). Advantages and disadvantages of different harvesting methods were highlighted.

(Kumar and Singh, 2019) indicated the EROI as the main metric for energy analysis of biodiesel biorefinery, highlighting that the literature has reported values spanning in a wide range (from near-zero to 4.3, but with a peak of even 8.35) due to the different assumptions (e.g., model scope, functional unit, system boundaries, co-product allocation). Overall, the low values of EROI of standalone microalgal biodiesel production indicated that the current technology is energetically unsustainable, while integrated biorefinery routes (for example, including wastewater treatment and biogas production) are promising.

De Souza et al. (2019) observed that electricity consumption was the major contributor to the environmental impacts in the production of either astaxanthin or biogas. Other main findings from LCA studies included a lower GWP of raceways compared to air-lift PBR, a decrease of water requirement by 90% and the elimination of the need for all the nutrients except phosphate by using sea/wastewater, GHG emissions of algal biojet fuel reduced by 76% compared to those of conventional jet fuel, GWP and fossil energy requirement savings of 42% and 38% compared to fossil-derived diesel, centrifugation and flocculation producing the highest and lowest impacts, respectively, among harvesting techniques. The most used software tools for LCA of microalgae-based systems were found to be CMLCA, GaBi, SimaPro, and GREET model.

Mishra et al. (2019) reported values of NER, defined as input over output energy, higher than 1 for microalgal bioenergy systems, with the exception of a promising process scheme with anaerobic digestion and pre-treatment (NER of 0.71), which also led to negative GHG emissions (−60.84 g CO_{2-eq} MJ^{-1}). Valorising by-products (pyrolysis and gasification of lipid-depleted residual microalgae biomass) led to net energy balances as low as ~0.6.

Wu and Chang (2019) described four microalgal biorefinery product chains through the LCA and TEA methods, by prospecting a process optimization by a multi-objective combinatorial approach that maximizes the NPV and minimizes the eco-indicator 99.

Ubando et al. (2019) reported values of GWP of thermochemical processes for bioenergy products from microalgae equal to ~0.045, 0.025-0.055, 0.03 kg $CO_{2\text{-eq}}$ MJ^{-1} for pyrolysis, liquefaction, and gasification, respectively.

Schade and Meier (2019) compared different microalgae cultivation systems (type, climatic zone of installation, and algal species) for human nutrition products, including two LCA studies with primary data at pilot scale. The biomass productivity in terms of land use ranged widely from 0.1 to 3.0 m^2 y kg^{-1}, and the corresponding CED ranged from 59 to 120 MJ kg^{-1}, showing clearly a beneficial effect of solar radiation and temperature on the process performance. Heating was by far the major item in electricity consumption, followed by aeration and CO_2. In turn, electricity consumption dominated almost all the environmental impact categories (ReCiPe Midpoint 2016), but water consumption played a major role as well, especially in ORPs. Overall, performances of different ORPs and PBRs were rather mixed. Therefore, the best option needs to be found in every single case based on site characteristics, microalgae species and target products.

By reviewing LCA studies for the use of microalgae residue to produce polylactic acid (PLA), Bussa et al. (2019) found that information on temporal boundaries is generally lacking. The reviewed LCA studies were based on different functional units (related to dimensions or to capacity of packaging) and different LCIA methods, while they were more homogeneous in the simulation of cradle-to-grave boundaries and in the use of data from the literature. The treatment of multi-product processes was based mainly on system expansion, but several studies adopted allocation. The results suggested that, in the PLA production, microalgae could reduce significantly land use and terrestrial ecotoxicity, as well as reduce to some extent eutrophication, human toxicity, photochemical oxidant formation and acidification potential. On the other hand, microalgae might worsen the performance of PLA compared to conventional plastics in other impact categories such as global warming, marine ecotoxicity, ozone layer depletion, and energy demand.

Koyande et al. (2019) reported that GHG emissions assessed for microalgal biofuel production with HTL were −220 $gCO_{2\text{-eq}}$ MJ^{-1}. Many values of NER were reported, e.g., 0.2-8.34 for the cultivation step only, 0.82 and 1.73 for cultivation and harvesting (the higher value estimated for a system including a photovoltaic panel), and 0.35-0.68 as energy demand (i.e., the reciprocal of the common definition, which is also preferred in this paper) for biofuel production.

Overall, the data and conclusions of previous review papers discussed in this section show that geographic location, technologies for upstream and downstream processes, process route, products, co-products (if any), and model assumptions affect greatly LCA results, which span in wide ranges. Therefore, the sustainability of microalgal biorefineries is strongly uncertain. However, the integrated approach of multi-product biorefinery is promising. In the sustainability evaluations, a standardization in the methodology is a minimum requirement to make comparisons, at least among process systems with similar final products. For example, a homogenization in the functional unit and unique definitions of performance metrics such as the NER are needed. Another crucial point is represented by the scale of the process used as source of primary data. A realistic description of current technologies requires a special focus on assessments based on the largest scale (highest TRL) available plants, which is lacking in the literature. The following sections of the present paper are devoted to fill this gap.

5. Pilot plants in environmental assessments

The main features of microalgal pilot plants providing primary data for LCA are described in Table 2. Different algal species were cultivated, without a predominance of any of them. Closed systems (PBR) were used in most cases, but open systems (ORP) were considered as well. A minimum volume of cultivation reactors approximatively of 100 L was considered in the selection of LCA studies. Most plants were of medium-low size, while large scale plants were a few. The culture medium was based on seawater in most cases, followed by freshwater, while a few pilot plants used wastewater. However, nutrients were added almost in all cases. For carbon supply, air or CO_2-enriched air were used in most cases; pure CO_2 or flue gas were adopted in some cases. The pilot plants were installed and tested mostly in European countries, followed by American locations. Harvesting was mostly performed via centrifugation, either standalone or in combination with other techniques, but flocculation was adopted in several case studies. Drying was performed in some cases.

The biomass productivity was provided either as volumetric or areal. However, in most cases data were insufficient to switch from one to the other. In volumetric terms, biomass productivity spanned from 0.0011 to 1.5 $g_{dw}\ L^{-1}\ d^{-1}$, with most values in the middle part of the range. In areal terms, the biomass productivity ranged 3 to 33 $g_{dw}\ m^{-2}\ d^{-1}$, with almost 20 $g_{dw}\ m^{-2}\ d^{-1}$ on average. Almost all the pilot installations were not provided with downstream processes, i.e., they produced wet or dry biomass, but not other products. In two exceptions bioactive compounds were extracted (via chemical methods). Many plants were tested for several months, but in three cases the testing period was lower than 1 month. Note that some data were not available in the reviewed studies (“N.A.” in Table 2), especially for biomass concentration, areal-to-volumetric productivity conversion, and testing time.

Examples of pilot systems of microalgae cultivation assessed by LCA are shown in Fig. 11.

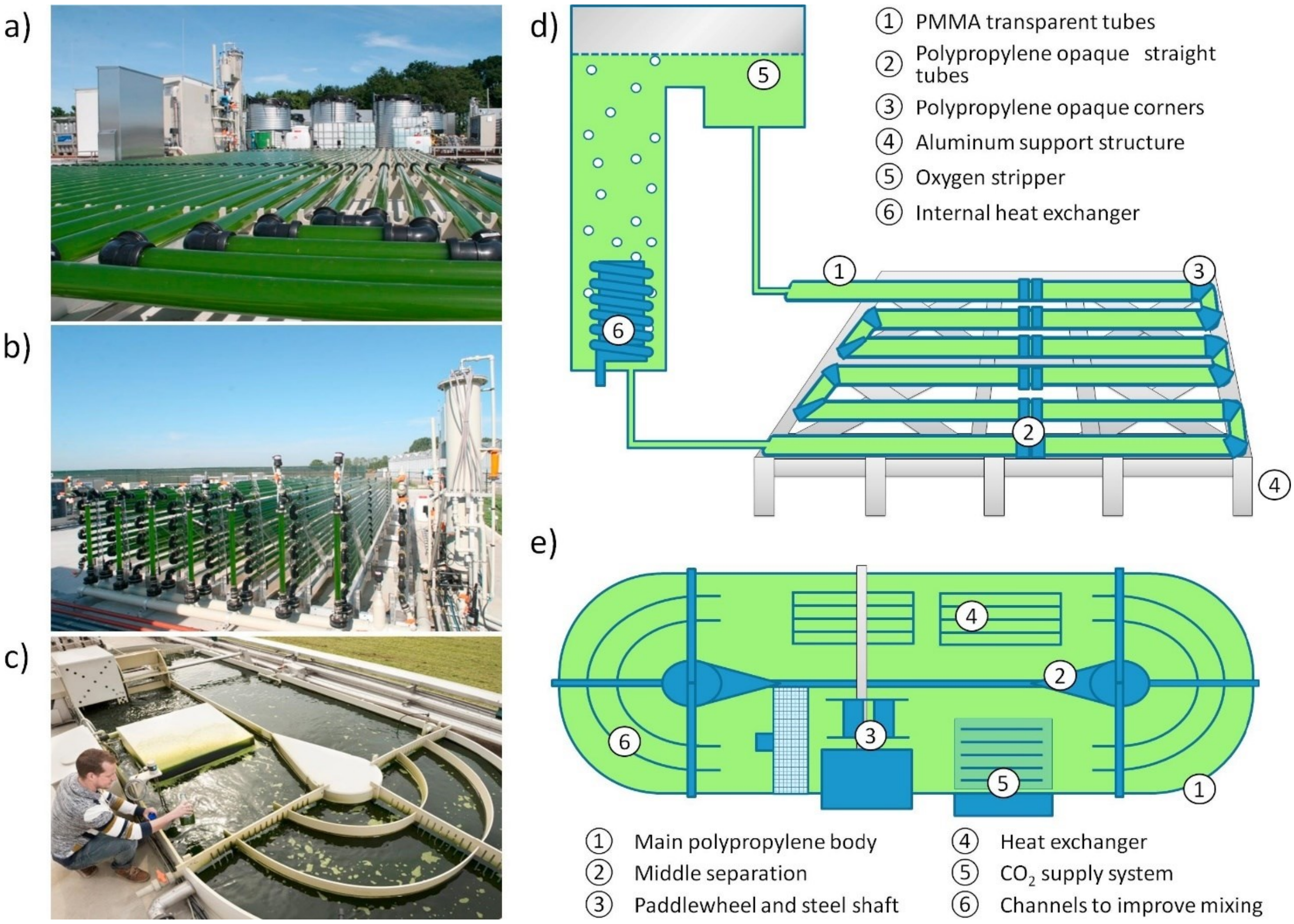


Fig. 11. Pilot-scale systems for microalgae (*Nannochloropsis sp*.) cultivation: (a) Horizontal tubular PBR, (b) vertically stacked (fence type) tubular PBR (c) ORP, (d) schematics of the main components of the tubular reactors and (e) schematics of the main components of the ORP (excluding pumps, compressors and nutrient dosage tanks). Reproduced from Pérez-López et al. (2017), with permission from Elsevier, copyright 2017.

Table 2. Main features of microalgae-based pilot plants. Only primary data are reported (secondary or tertiary data are excluded, e.g., scaled-up literature's data). "N.A." means "not available".

Algal species	**Cultivation system (where not specified, outdoor); biomass conc.** **[kg_{DW} m^{-3}]**	**Growth medium**	**CO_2 source**	**Location**	**Harvesting system; biomass conc.**	**Biomass productivity**	**Downstream phases**	**Main product; co-products**	**Testing time**	**Reference**
1. *Alexandrium minutum* 2. *Karlodinium veneficum* 3. *Heterosigma akashiwo*	Bubble column PBRs, 297 L (99 L per algal species): a) indoor with temperature-controlled room at 20°C ± 1°C and irradiated with fluorescent lights or b) outdoor; 1. a) 1.25, b) 0.97 2. a) 1.18, b) 1.03 3. a) 1.2, b) 0.98	Filtered seawater with L1-enriched medium without added silicates	Pre-filtered air	Barcelona (Spain)	Centrifugation; ~98.5% dw (wet pellet)	N.A.	X	Biomass	~ 160 days	Sevigné Itoiz et al. (2012)
N.A.	PBR, 12 m^3, 10 m^2; N.A.	Tap water with modified Chu medium	Air	Curitiba (Brazil)	Flocculation (NaOH or $FeCl_3$), press-filtration and spray drying; N.A.	1.5 g L^{-1} d^{-1}	X	Biomass	9 days	Silva et al. (2015)
Nannochloropsis sp.	1. Horizontal PBR, 560 L; 0.6-2.5 2. Vertically stacked PBR, 1060 L; 0.4-1.9 3. ORP, 4730 L; 0.5 Heather/chiller for T-control at 20-30 °C.	Seawater (chlorinated, microfiltered, and de-chlorinated by activated carbons) with nutrients	CO_2-enriched air	Wageningen (Netherlands)	Microfiltration and centrifugation; 22% dw	1. 0.067-0.655 g L^{-1} d^{-1} 2. 0.079-0.569 g L^{-1} d^{-1} 3. 0.011-0.057 g L^{-1} d^{-1}	X	Biomass	~2.5-5 months	Pérez-López et al. (2017)
Desmodesmus subspicatus	ORP, 8000 L (4 tanks of 2000 L each) with air lift or paddle wheels; 1. 0.6 2. 1.12	1. Wastewater (UASB treatment of toilet wastewater) 2. NPK solution	Air	Laguna (Brazil)	Flocculation with NaOH or electroflotation (Al or Fe electrode), filtration or centrifugation, and drying in tray oven; N.A. (probably water content is ~0 after drying). *Separation and drying were*	1. 17 g m^{-2} d^{-1} (0.1125 g L^{-1} d^{-1}) 2. 28 g m^{-2} d^{-1} (0.15 g L^{-1} d^{-1})	X	Biomass	N.A. (10 days per batch)	Schneider et al. (2018)

					studied on a minor scale.					
Acutodesmus obliquus	Greenhouse systems: 1. Tubular PBRs, 250 L, 4.2 m^2, water-cooling by sprinkling; 2.1 2. Mesh ultra-thin layer PBRs, 100 L, 4.84 m^2, with water-cooled double bottom; 6.6	Water with added minerals; 1/2 Tamiya medium for inoculum	CO_2 injection by magnetic valve (automatically regulated by pH measurement)	Nuthetal (Germany)	Centrifugation; N.A.	1. 0.15 g L^{-1} d^{-1} 2. 0.47 g L^{-1} d^{-1}	X	Biomass	1. 12 batches 2. 7 batches 14 days per batch. Experiments during a period of 3 years	Sandmann et al. (2021)
Nannochloris sp., *Nannochloropsis* sp.	ORP, ~1000 m^2; < 1	Waste seawater (from a power plant) with fertilizer, groundwater to mediate pond salinity	Waste flue gas with 13-14% CO_2	Ashkelon (Israel)	Centrifugation; 20% dw	3 g m^{-2} d^{-1}	X	Biomass	N.A., but the value of productivity is an annual average	Passell et al. (2013)
1. *Staurosira* sp. 2. *Desmodesmus* sp.	Hybrid system with PBR (25 m^3, 186 m^2, horizontal serpentine, airlift driven) and ORP (60 m^3, 421 m^2); N.A.	Filtered seawater with fertilizers	CO_2 at purity > 99%	Kona, Hawaii (U.S.)	Settling, centrifugation, and ring drying; 90%	1. 19 or 33 g m^{-2} d^{-1} 2. 23 g m^{-2} d^{-1}	X	Biomass	~17 months	Beal et al. (2015), Huntley et al. (2015)
Scenedesmus obliquus	ORP, 2500 L, 11 m^2; 0.43	Freshwater with livestock wastewater ($0.5\%_{v/v}$)	Pumped air	Campania (Italy)	Flocculation and centrifugation; 200 kg_{DW} m^{-3}	0.047 g L^{-1} d^{-1}	X	Biomass	N.A.	Jez et al. (2017)
Scenedesmus dimorphus	ORP, 65232.5 m^2; 0.5	Freshwater	Air	Mysore (India)	Flocculation with $FeCl_3$; N.A.	6 g m^{-2} d^{-1}	X	Biomass	3 months	Togarcheti et al. (2017)
Phaeodactylum tricornutum	Bubble column PBR, 800 L, thermoregulated by a cooling equipment using freshwater; 0.96 (maximum)	Walne medium in seawater for inoculum, seawater with silicate for cultivation	Air	Concepción (Chile)	Centrifugation; 150 kg_{DW} m^{-3}	0.13 g L^{-1} d^{-1} (maximum)	X	Biomass	14 days	Branco-Vieira et al. (2020, 2018)
Nannochloropsis oceanica	Green Wall Panel (GWP®) PBR with air bubbling, 28.1 m^2, 1.4 m^3, cooling by heat exchanger and chiller (T<27 °C); 1.6	Seawater with nutrients	CO_2-enriched air	Sesto Fiorentino (Italy)	Centrifugation; 20% dw	N.A.	X	Biomass	< 1 month	Gaber et al. (2021)
Tetraselmis suecica	Indoor vertical bubble column PBR, 80 L, 20 °C, 12 h light per day with continuous, cool white and	Filtered seawater with nutrients	CO_2-enriched air from a power plant	N.A. (probably Santiago de Compostela, Spain)	Centrifugation; N.A.	N.A.	Three-step extraction with solvent: 1) hexane, 2) methanol and KOH after washing with ethanol, and 3) methanol aqueous	Bioactive compounds: 1) lipid fraction (45% PUFAs), 2) α-tocopherol,	60 days	Pérez-López et al. (2014b)

	fluorescent light sources; N.A.						solution. After each extractive process, algal paste was centrifuged.	chlorophyll, and β-carotenoid, and 3) polyphenols; algal residual paste		
Haematococcus pluvialis	Indoor airlift PBR, two-stage process, 2000 L (2 tanks), internally illuminated (16:8 regime); N.A.	River/rain water (purified by RO and UV) with nutrients (differing in concentration and composition for the two culturing stages)	Air	Louvain (Belgium) for cultivation, Claregalway (Ireland) for extraction	Settling (i) between growth and stress stage, and (ii) after stress stage, followed by centrifugation and spraydrying; 95% dw	N.A. (0.005 $g_{astaxanthin}$ L^{-1} d^{-1})	Supercritical CO_2 extraction of astaxanthin	Nutraceutical oleoresin with 10% astaxanthin content	N.A.	Pérez-López et al. (2014a)
Haematococcus pluvialis	Multi-stage cultivations, including green and red phases: 1. Outdoor Green Wall Panel (GWP) PBR, 0.1 m^3, illuminated by LEDs; 1.16 (red phase) 2. Indoor Flat Panel Airlift (FPA) PBR, 93 m^3, 0.11 ha, illuminated by LEDs and ventilated for T-control; 3.75 (red phase) 3. Outdoor Unilayer Horizontal Tubular (UHT) PBR, 93 m^3, 0.24 ha, cooling by freshwater spraying; 0.88 (red phase)	Freshwater with nutrients	Pure CO_2 (air for mixing)	1. Montpellier (France) 2. Stuttgart (Germany) 3. Lisbon (Portugal)	1. X 2. X 3. Microfiltration and centrifugation; 10-20% dw	1. 0.21 g L^{-1} d^{-1} 2. 0.75 g L^{-1} d^{-1} 3. 0.07 g L^{-1} d^{-1} (red phase)	X	Biomass	N.A.	Onorato and Rösch (2020)

6. LCA with primary data from microalgae pilot systems

6.1. Methods

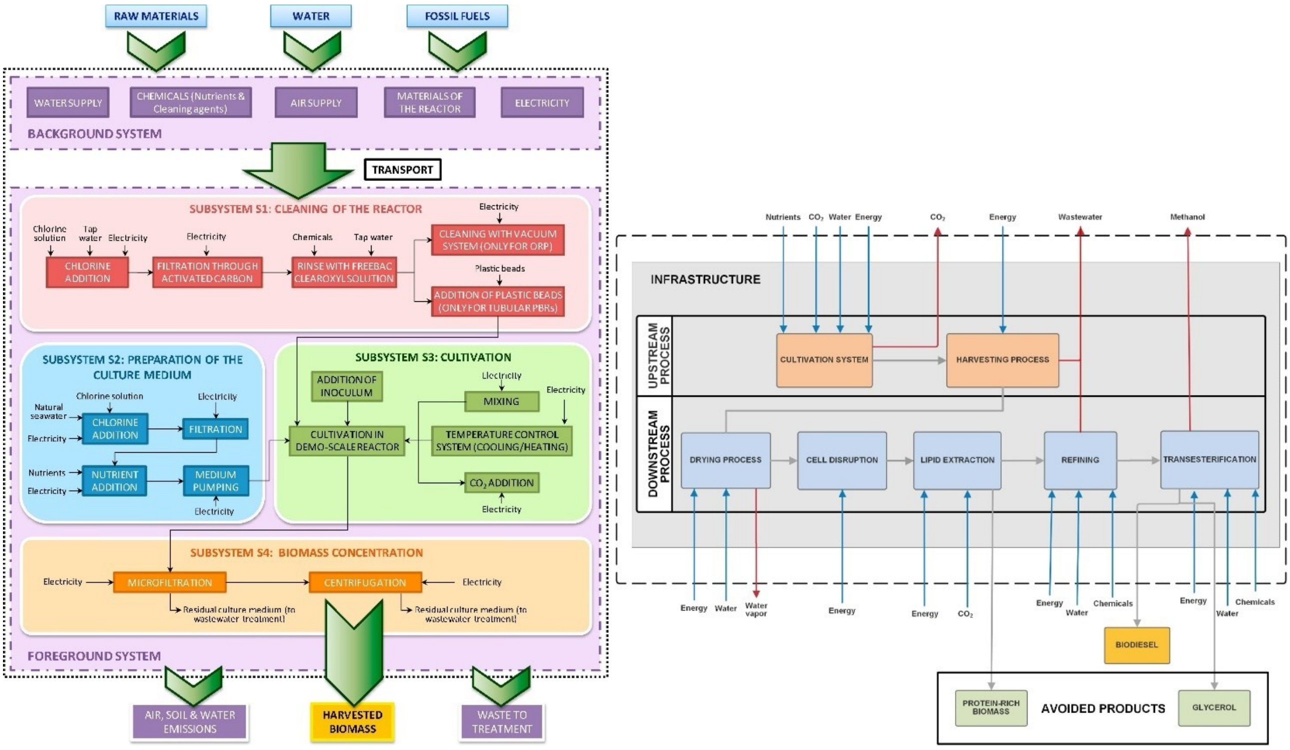
The microalgal systems assessed by LCA with primary data from pilot plants are described in Table 3. Out of the fourteen studies selected, biomass, biofuels, and high-value products were considered as main products in five, six and three LCAs, respectively. They are grouped in this order in Table 3 and throughout the paper. In the microalgal systems for biofuels and high-value products, several co-products have been considered, depending on the process route assessed.

The goal of the studies is the assessment of potential environmental impacts and energy consumption of microalgal-based production systems. Specifically, several LCA studies compared different systems, e.g., in terms of cultivation reactors, cultivation media, downstream processes, or plant location. In some LCA study, a comparison with equivalent products was included (i.e., conventional (bio)fuels). The modelling framework is attributional LCA.

The scope of the studies is described across various columns of Table 3 and Table 4. The functional unit (FU) was 1 kg of dry biomass in most studies devoted to biomass production. In the case of biofuel products, 1 kg or 1 MJ of them was chosen as FU. When systems for high value biochemicals production were assessed, the FU was chosen with reference to the mass of microalgae or of the compound. There are also LCAs that used the size of the plant as FU. This latter choice bypasses the issue of multi-functionality treatment (allocation, system expansion), but, to the authors' opinion, makes comparisons meaningless, unless those ones between systems with the same products.

In 40% of the LCA studies, some scale-up of the primary data was performed, and projected/optimized scenarios were simulated. The comparison of Table 2 (especially the *Main product; co-products* column) with Table 3 (especially the *System boundaries* column) highlights that the products of real pilot plants (biomass in most cases) often differ from the products of the systems evaluated in the LCA, where the boundaries are extended by simulating downstream processes that do not exist in the real pilot installation. Therefore, most LCA studies treated the foreground product system only partially with primary data from pilot plants, while a relevant part of it regarding downstream processes was modelled by literature data. The cradle-to-gate boundaries are used in all studies, apart from one. Two examples of product system with its boundaries are provided in Fig. 12.

Infrastructure (i.e., construction materials) was considered in most LCAs, while transportation of materials is included in fewer studies. The temporal boundary of the LCA was often omitted. When explicitly declared, the equipment lifespan was between 10 and 40 years. The geographic boundary reflects the location of the pilot installation, but additional scenarios were often simulated by changing the electricity grid mix. Finally, the yearly operating time of the plant was omitted in most studies. Otherwise, it was around 300 days per year.



(a) (b)

Fig. 12. Product system (foreground and background) for (a) the cultivation of *Nannochloropsis sp*. in pilot-scale reactors, and (b) the biodiesel production from *Phaeodactylum tricornutum*. Reproduced from (a) Pérez-López et al. (2017), with permission from Elsevier, copyright 2017; (b) Branco-Vieira et al. (2020), with permission from Elsevier, copyright 2020.

Key methodological elements of the reviewed LCA studies are reported in Table 4. SimaPro and Ecoinvent were the most used LCA software and database, respectively. Multi-functionality, which occurs in the product systems for biofuels or biochemicals production, was treated mostly by system expansion (avoided products), while allocation was applied in some cases. Different methods were used for impacts assessment. CML 2001 was the most used, followed by ReCiPe. Environmental impacts were evaluated ad midpoint in most cases (10 LCAs), while endpoint assessments were performed in fewer studies (4 LCAs). In almost all cases, sensitivity, scenario or uncertainty analyses were performed, thus broadening the range of results and their reliability.

Overall, by analysing Table 3 and Table 3. Features of systems evaluated in LCA studies based on primary data from pilot plants. “N.A.” means “not available”.Table 4 we can observe many differences in the methodologies applied in the reviewed LCA studies. Several information was even not available. Heterogeneity of methods and lack of transparency is an issue for the analysis of results, as it will make comparisons difficult.

Table 3. Features of systems evaluated in LCA studies based on primary data from pilot plants. “N.A.” means “not available”.

Goal	**Functional Unit**	**Scale-up of primary data**	**System boundaries**					**Operating time [day y^{-1}]**	**Reference**
				Infrastructure	**Transport**	**Temporal (or equipment lifespan) [years]**	**Geographic**		
To determine the principal environmental and energy impacts in the production of marine microalgal biomass under artificial (indoor) and natural (outdoor) conditions in a bubble column PBRs pilot plant.	1 kg of dry biomass	X	Cradle-to-gate for biomass production	✓	X	10	Spain, energy mix with 57% fossil fuel energy	N.A.	Sevigné Itoiz et al. (2012)
To assess the environmental impacts of microalgae cultivation in tubular compact PBRs at industrial scale.	N.A. (biomass product)	X	Cradle-to-gate for biomass production	✓	✓	N.A.	Brazil, electricity from hydropower	N.A	Silva et al. (2015)
To assess three different reactor configurations for the pilot-scale production of *Nannochloropsis sp.* in three periods of the year (summer, fall and winter), identifying environmental hotspots.	1 kg of dry biomass	Additional hypothetical scenarios without T-regulation system (pilot) and with ~70% reduction in energy consumption (large-scale)	Cradle-to-gate for biomass production	✓	✓	10 or 20, depending on properties and function of building materials	The Netherlands	~304	Pérez-López et al. (2017)
To evaluate the potential environmental impacts of microalgae biomass production and to determine the best strategy for reducing them. The use of wastewater or NPK in the cultivation stage (ORP) is followed by several separation methods and drying in order to find a more environmentally friendly metod.	8000 L raceway pond	X	Cradle-to-gate for biomass production	X	X	N.A.	Brazilian electricity mix (12.46% fossil fuels, 80.29% hydropower, 2.88% nuclear, 4.32% renewables)	N.A.	Schneider et al. (2018)

To compare two designs of PBRs (a reference tubular PBR vs. an innovative prototype reactor MUTL) for microalgae production with defining the environmental hotspots of the systems and perspectives for the improvement.	1 kg of dry biomass or 1 mmol of specified antioxidant capacity	X	Cradle-to-gate for biomass production	X	X	N.A.	N.A.	N.A.	Sandmann et al. (2021)
To assess algae biodiesel by using data from commercial partners to capture the impacts from current commercial capabilities. The results were compared with soy biodiesel and low sulfur diesel.	1 MJ produced by fuel (biodiesel) combustion in a CIDI vehicle	1:101 in culture area for future scenario, where scaled-up data were optimized. Oil extraction is modelled at large-scale with data estimated from batch-scale source data.	Pond-to-wheels for biodiesel production and use, simulating by extrapolation from batch-scale data and by the GREET 1_2011 model downstream processes of wet extraction of oil, transesterification for oil-to-biodiesel conversion, and combustion in CIDI vehicle. Co-products: low value lipids, residual dry biomass (oilcake), and glycerine.	X	X	N.A.	Average U.S. electricity grid or average German electricity grid (the share of fossil fuels is about 55%, i.e., 15% less than the average U.S. grid)	N.A.	Passell et al. (2013)
To assess environmental impacts associated with ten case studies for biofuels and feed production from microalgae.	1 ha of facility area for cultivation and processing	1:1341 in culture volume	Cradle-to-gate for biocrude production, simulating by experimentally derived data or literature data downstream processes of hexane extraction, Valicro thermochemical conversion, HTL, OpenAlgae's extraction, fermentation, catalytic hydrothermal gasification, and combined heat and power in different scenarios. Co-products: protein-rich and omega-3-fatty-acid rich animal feed, and ethanol. Filter pressing is simulated after settling in an alternative harvesting and dewatering configuration assessed. The carbon source assumed for the LCA was a 94% CO_2 waste stream.	✓	✓	30	Hawaii or Texas (*when not available, data for a global average excluding European Union (RoW) have been used*).	347	Beal et al. (2015)
To evaluate the hot spots in site-specific production chain of biodiesel from terrestrial and microalgae feedstock, and to compare their impacts with first generation biofuels.	1 kg of produced oil	X	Cradle-to-gate for refined oil production, simulating by literature data downstream processes of solvent extraction of microalgae oil, and stripping recovery for microalgae oil/hexane separation. Co-product: cake.	✓	X	10 for pond and 20 for centrifuge	Italian medium voltage production, at grid	275	Jez et al. (2017)

To analyze the life cycle energy requirements and environmental impacts of microalgae-based biodiesel production process under multiple scenarios.	1 kg of biodiesel	X	Cradle-to-gate for biodiesel production, simulating by literature data dewatering by spray drying and mechanical drying, and downstream processes of lipid extraction (with hexane), conversion to biodiesel (transesterification), and bioelectricity generation from biogas; and simulating by lab-scale data anaerobic digestion of residual biomass. Additional scenarios simulated by literature data.	X	X	N.A.	India, hard coal energy source for electricity; U.S. average grid electricity for additional scenarios	N.A.	Togarcheti et al. (2017)
To assess the production of biodiesel from *P. tricornutum* cultivated in an industrial plant facility.	1 MJ of biodiesel	1:10,000 in culture volume	Cradle-to-gate for biodiesel production, simulating by literature data downstream processes of freeze-drying, cell disruption by ball mill, lipid extraction by supercritical CO_2, refining, and transesterification. Co-products: protein-rich biomass, glycerol.	✓	✓	30	Chile	351	Branco-Vieira et al. (2020)
To assess and compare the environmental impacts of Total Fatty Acid production with *Nannochloropsis oceanica* considering scenarios of upscaled production in novel Green Wall Panel PBRs at different sites in Italy (Tuscany and Sicily), identifying the ecological hot spots.	1 kg of TFA	1:4500 in culture volume, up-scaled data were optimized	Cradle-to-gate for TFA production, simulating by literature data downstream processes of cell disruption by homogenization, TFA extraction by solvent (MTBE), 3-phase separation, and solvent vaporization for recycle. HTL was included in additional scenarios (co-products: biocrude, nutrients).	✓	N.A.	N.A.	Italian or Norwegian electricity mix	240 or 330	Gaber et al. (2021)
To identify the environmental profile of the production of five bioactive compounds (PUFAs, α-tocopherol, chlorophyll, β-carotenoid, and polyphenols) from *Tetraselmis suecica*.	1 kg of dry biomass	X	Cradle-to-gate for bioactive compound production, simulating by literature data downstream processes of the residual algal paste, i.e., settling and centrifugation, anaerobic digestion, combustion for electricity production and fertilizer (digestate) production.	✓	✓	N.A.	Spain for production of chemicals, equipment, materials and electricity; Chile for nitrate production	N.A.	Pérez-López et al. (2014b)
To perform a comparative assessment of the environmental impacts associated with the production of *H. pluvialis* astaxanthin for nutraceutical or pharmaceutical uses at both lab and pilot scale in airlift PBRs with artificial illumination.	800 g astaxanthin	X	Cradle-to-gate. Additional scenarios simulated by literature data.	✓	✓	40 for PBRs	Belgian and Irish grid	N.A.	Pérez-López et al. (2014a)

To evaluate the environmental impact of three different PBRs located in three different regions with/without artificial light for the production of *H. pluvialis* at 80–85% dw or astaxanthin during a period of one year.	1 kg of dry biomass or 1 kg of astaxanthin	1. 1:930 in culture volume for GWP 2. X for FPA 3. X for UHT	Cradle-to-gate for biomass or astaxanthin production, simulating by literature data either (1 and 2) complete harvesting with microfiltration, centrifugation and spraydrying, and astaxanthin extraction via supercritical CO_2, or (3) spraydrying and astaxanthin extraction, depending on the availability of primary data associated to three different PBR systems: 1. GWP or 2. FPA or 3. UHT Pure astaxanthin is assumed from extraction. Co-products in case of astaxanthin production: residual biomass substituting animal feed; or CO_2, nutrients and electricity recycled from anaerobic digestion.	✓	X	10 for PBRs and 20 for other equipment	1. French grid mix 2. German or French grid mix 3. Portuguese grid mix	1. 310 (green phase), 225 (red phase) 2. 310 (green phase), 225 (red phase) 3. 330	Onorato and Rösch (2020)

Table 4. Key elements of the methods used in LCA studies based on primary data from pilot plants. “N.A.” means “not available”.

Software	**Database for background**	**Multifunctionality: Expansion (avoided products) / Allocation**	**Method for impacts assessment**	**Endpoint**	**Sensitivity / scenario / uncertainty analysis**	**Reference**
SimaPro 7.1.8	Ecoinvent (version N.A.)	X	CML 2001, CED 1.4	X	Net energy balance in five scenarios with increasing lipid content (25% upwards at intervals of 10%) and decreasing energy consumption (reduction by 50% for each subsequent scenario starting from the base case)	Sevigné Itoiz et al. (2012)
SimaPro 7	N.A	X	Ecological footprint, CML 2001, TRACI	✓	X	Silva et al. (2015)
SimaPro 8	Ecoinvent (version N.A.)	X	CML 2001, CED	X	8 scenarios based on cultivation method and season, plus hypothetical scenarios of energetically efficient pilot- and large-scale plants	Pérez-López et al. (2017)
SimaPro 8.4.1.0	Ecoinvent 2.1	X	ReCiPe 1.06	✓	- Ten scenarios depending on cultivation medium, separation, and drying - Uncertainty analysis with Monte Carlo simulation	Schneider et al. (2018)
SimaPro 8.2.0.0	Ecoinvent 3.1, Agri-footprint	X	IMPACT 2002+ 2.21	✓	Uncertainty analysis by Monte Carlo simulation	Sandmann et al. (2021)
SimaPro 7.2	Ecoinvent 2.0	Allocation based on energy content, or expansion	N.A.	X	- Scenario analysis with 1) base case and 2) estimated future case of more efficient larger scale production; - Sensitivity analysis (further scenarios) for base case to biomass productivity, and for future case to a) grid, b) multi-functionality treatment, c) energy consumption by centrifuge, d) energy consumption by paddle wheel, e) energy consumption by belt filter press, f) algae oil / biomass ratio.	Passell et al. (2013)

OSMOSE	Ecoinvent 3.1	X (The chosen FU does not require multifunctionality treatment)	IMPACT 2002+, ReCiPe	✓	- Ten scenarios for both geographical locations (Hawaii or Texas) including five different process schemes and differing in several parameters (mixing method in the pond, nitrogen dosage, biomass productivity, electricity source) - Sensitivity analysis (for five of the previous scenarios) by considering two additional values (one less favourable and one more favourable) for several parameters	Beal et al. (2015)
SimaPro 7.3.3	Ecoinvent 2.2	Expansion	ReCiPe 2008, CED	X	- Scenario analysis: 1) conventional electricity; 2) solar energy; 3) electricity from biogas produced by algae cake. - Uncertainty analysis by Monte Carlo method.	Jez et al. (2017)
GaBi 6.5.1.8	Ecoinvent, Peter Eyerer, European Life Cycle Database, Plastics Europe (versions N.A.)	Allocation	CML 2001	X	Three scenarios with different biomass productivity, mode of culture mixing and type of energy source.	Togarcheti et al. (2017)
SimaPro 9	EcoInvent 3.3, ELCD 3.2, Agri-footprint 3.0	Expansion	ReCiPe 2016 (H) V1.00	X	Sensitivity analysis with ±10% perturbation of various process parameters	Branco-Vieira et al. (2020)
OpenLCA 1.7	Ecoinvent 3.4	Expansion	ILCD Midpoint 2011	X	Scenario analysis: 1) Baseline; 2) Nutrient recycling and energy credit via HTL; 3) 2 + renewable energy. Two locations: a) Sicily; b) Tuscany.	Gaber et al. (2021)
SimaPro 7.3	Ecoinvent 2.0	Expansion for avoided electricity and fertilizers, Allocation by mass for various co-products	CML 2001 2.04	X	Scenario analysis: 1) Base case with use of the algal paste for biogas production; 2) Five alternative scenarios with different nitrogen sources (including digestate and algal paste) for cultivation	Pérez-López et al. (2014b)
SimaPro 7.3	Ecoinvent 2.0, IDEMAT 2001	Expansion	CML 2001	X	Scenario analysis: 1) Baseline (annular PBR with artificial light, producing 800 g astaxanthin); 2) annular PBR with sunlight, producing 400 g astaxanthin; 3) flat-panel PBR with artificial light, producing 800 g astaxanthin; 4 flat-panel PBR with sunlight, producing 400 g astaxanthin	Pérez-López et al. (2014a)
OpenLCA 1.6	Ecoinvent 3.3	Expansion	ReCiPe (midpoint (H)) 2014	X	Scenario analyses by changing PBR type, using either French or German grid mix, four scenarios of residual biomass digestion (astaxanthin content and methane yield), and four scenarios (astaxanthin content) of residual biomass use as animal feed, combined in a total of either 16 or 15 scenarios of expanded systems for biomass digestion or feed	Onorato and Rösch (2020)

6.2. Results

The LCA results of the selected studies are presented in the following sub-sections, which are differentiated in terms of main final product of the microalgae-based system evaluated. Of course, several co-products may be present in the assessed systems. LCI and LCIA results from different studies are heterogeneous due to differences in the adopted methodologies, in the presentation of data, and in the products of the process schemes analysed. Therefore, a comprehensive comparison among studies is not feasible, not even in the case of same or similar main product(s). Within these limits, LCA results from the reviewed papers are summarized, analysed, and discussed in the following, including some detailed examples of LCIA outcomes.

6.2.1.Biomass

Biomass is the final product in five LCA studies, three of which regarded PBRs for cultivation (Sandmann et al., 2021; Sevigné Itoiz et al., 2012; Silva et al., 2015), one dealt with open ponds (Schneider et al., 2018), and one compared both systems (Pérez-López et al., 2017) (Table 2). Infrastructure was included in three cases (Pérez-López et al., 2017; Sevigné Itoiz et al., 2012; Silva et al., 2015) (Table 3).

LCI

Focusing on the studies with the FU of 1 kg of dry biomass, Sevigné Itoiz et al. (2012) reported 0.2 and 0.3 kg kg^{-1} for construction materials, 0.85 and 1.06 m^3 kg^{-1} for water consumption (seawater and water for maintenance washing, producing similar amounts of wastewater output), ~7-9 g kg^{-1} for nutrients, and energy consumption of 139-908 MJ kg^{-1} (i.e., 38.6-252.2 kWh kg^{-1}), where lower values were for outdoor cultivation, while higher values were for indoor cultivation. In all cases, growing was the most energy-intensive stage, while dewatering via centrifugation played only a minor role. Pérez-López et al. (2017) reported a detailed LCI, including construction materials of ~0.4-9.0 kg kg^{-1}, 128-15984 L kg^{-1} for tap water (cleaning), 426-15628 L kg^{-1} for seawater, ~0.9-6.6 kg kg^{-1} for $NaNO_3$ (main additional nutrient), 2.31-22.7 m^3 CO_2, ~280-5600 kWh kg^{-1} for energy consumption (major contribution due to the cultivation stage, especially by the heating / cooling system). The minimum and maximum values of the ranges reported above regarded horizontal PBRs operated in summer and ORPs operated in winter, respectively. Vertically stacked PBRs (Fig. 11b) required material and energy flows slightly higher than horizontal PBRs (Fig. 11a). Overall, almost all the main inflows inventoried by Pérez-López et al. (2017) were significantly higher than those by Sevigné Itoiz et al. (2012). Pérez-López et al. (2017) inventoried output wastes, consisting of construction materials (to landfill) and wastewater from cleaning and biomass concentration (to treatment plant).

Some general considerations can be done from studies with different (or even not available) functional units. In Silva et al. (2015), concrete, steel and PVC are the most used construction materials (~12,000, 2600 and 2200 kg, respectively, probably indicating the whole mass of the plant). In Schneider et al. (2018) sodium hydroxide (for flocculation) is the most used material after water (15.36 kg vs. ~6500 kg per batch in a 8000-

liter raceway), which produced the various effluents reported as output. Sandmann et al. (2021) compared tubular PBRs with mesh ultra-thin layer PBRs, showing that the second option consumes less water and materials (cleaning and sterilization agents) due to a higher productivity, and leads to a higher specific antioxidant capacity. Note that Sandmann et al. (2021) used 1 kg of dry biomass or 1 mmol of specified antioxidant capacity as FU for the assessed impacts, but did not report the inventory based on either of them (Table 1 in Sandmann et al. (2021) seems rather referred to an average batch of cultivation).

LCIA

Regarding the Life Cycle Impact Assessment (LCIA), a comparison is possible between the results by Sevigné Itoiz et al. (2012) and those by Pérez-López et al. (2017), who used the CML 2001 method for impacts evaluation (Table 4) along with 1 kg of dry biomass as FU (Table 3). The impacts were lower in the LCA by Sevigné Itoiz et al. (2012) compared to that by Pérez-López et al. (2017) for most categories, e.g., GWP of 22.9-153 vs. 214-4256 kg $CO_{2\text{-eq}}$ kg^{-1} biomass and human toxicity of 5.64-45.6 vs. 49.7-836 kg 1,4-DB_{eq} kg^{-1} biomass. Compared to them, Sandmann et al. (2021) reported intermediate values of GWP (189.40 and 473.53 kg $CO_{2\text{-eq}}$ kg^{-1} biomass for tubular PBRs and mesh ultra-thin layer PBRs, respectively), but it was evaluated by a different method (IMPACT 2002+).

The GWP estimated by Sevigné Itoiz et al. (2012) was 6 times lower in outdoor than in indoor conditions. The growing phase required the largest energy consumption due to pumping and air injection, and caused the largest impacts, contributing by more than 95% of all the categories in indoor conditions and by ~65%-90% in outdoor conditions (where the second impacting item was the bubble column PBR construction).

Silva et al. (2015) found that structural steel, transparent and brown PVC for PBRs and packaging of materials transport contributed by more than 85% of the total impacts analysed by various methods.

In Pérez-López et al. (2017) the total CED ranged ~3500-70,000 MJ kg^{-1} dry biomass, with energy consumption for temperature regulation causing the highest environmental burdens. The production of nutrients affected the categories of acidification (CML 2001) and non-renewable energy demand from biomass of primary forests (CED) by ~20% and 60%, respectively. Waste treatment (landfill for solid waste, discharge in sewage for wastewater) was included in the analysis, resulting into a relative contribution higher than 10% to some impact categories only in the case of cultivation in ORP. Fig. 13 reports the relative environmental profiles assuming the ORP operated under fall conditions as a reference. The environmental performance was affected by the cultivation system and season. However, the configuration was decisive only in fall and winter, while it led to mild effects in summer. Overall, horizontal PBRs presented slightly lower environmental impacts than vertically stacked PBRs, while the ORPs was characterized by the highest impacts. The environmental performance of tubular PBRs was less dependent on weather conditions, while the ORP may only be feasible during a limited period of the year. This outcome differs from previous LCAs, which suggested that ORPs have lower environmental impacts than PBRs. However, the geographical location may be crucial (note that the LCA study by Pérez-López et al. (2017) regarded a plant installed in the Netherlands, characterized by low sunlight intensity, high rainfall and moderate to low temperatures). The simulation of large-scale plants with optimized temperature regulation systems exhibited reductions of environmental impacts and energy demand up to 90%, showing important potential improvements in the process scale-up.

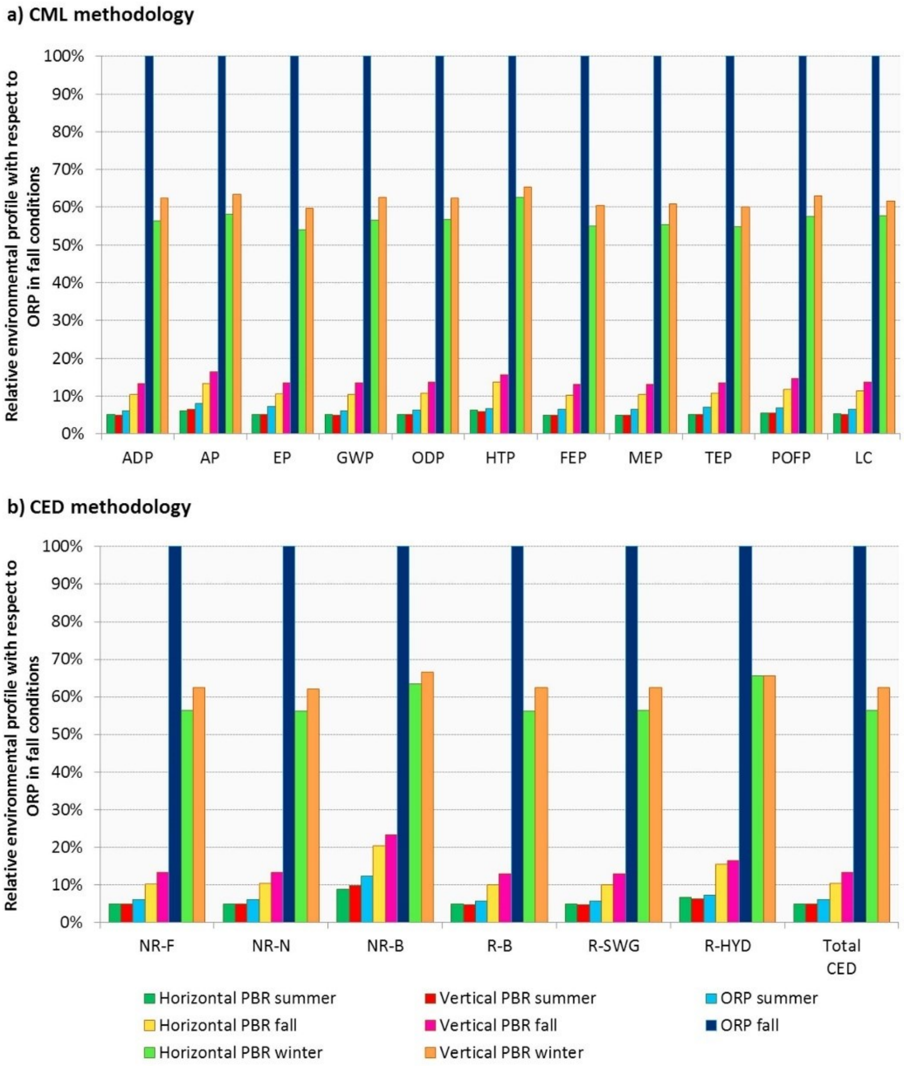


Fig. 13. Relative environmental profile of different cultivation configurations (horizontal PBR, vertically stacked PBR, and ORP, with cultivation in different seasons in the Netherlands followed by microfiltration and centrifugation) for the production of *Nannochloropsis* sp. algal biomass: impact categories of (a) CML methodology and (b) CED methodology. FU of 1 kg DW microalgal biomass. ADP – abiotic depletion; AP – acidification; EP – eutrophication; GWP – global warming potential; ODP – ozone layer depletion; HTP – human toxicity; FEP – freshwater ecotoxicity; MEP – marine ecotoxicity; TEP – terrestrial ecotoxicity; POFP – photochemical oxidants formation; LC – land competition; NR-F – non-renewable fossil energy demand; NR-N – non-renewable nuclear energy demand; NR-B – non-renewable energy demand from biomass of primary forests; R-B – renewable energy demand from food and agricultural sources; R-SWG – renewable energy demand from solar, wind and geothermal; R-HYD – renewable energy demand from hydropower; Total CEC – total cumulative energy demand. Reproduced from Pérez-López et al. (2017), with permission from Elsevier, copyright 2017.

GWP - Global warming potential; SOD - Stratospheric ozone depletion; IOR - Ionizing radiation; OFH - Ozone formation, human health; FPF - Fine particulate matter formation; OFT - Ozone formation, terrestrial ecosystems; TAC - Terrestrial acidification; FWE - Freshwater eutrophication; TEC - Terrestrial ecotoxicity;

FEC - Freshwater ecotoxicity; MEC - Marine ecotoxicity; HCT - Human carcinogenic toxicity; HNT - Human non-carcinogenic toxicity; LUS - Land use; MRS - Mineral resource scarcity (kg Cu eq); FRS - Fossil resource scarcity; WAC - Water consumption

Comparing different cultivation and separation scenarios, Schneider et al. (2018) did not find differences in the impacts by using air lift or paddle wheels. In contrast, using wastewater led to lower impacts than using the NPK solution. Despite both culture media led to carbon credits (net emission of ~–14 kg $CO_{2\text{-eq}}$), the use of wastewater avoided environmental impacts because (i) there were no added inputs in the medium and (ii) it avoided wastewater treatment. This resulted in a negligible endpoint impact, while the NPK solution totalized a score higher than 350 mPt, especially associated to damage to human health and natural resources. Electricity consumption was the most detrimental input for almost all impact categories. Regarding the dewatering stage, electroflotation caused fewer impacts than flocculation (~180 mPt vs. 280 mPt). However, the scenario with lowest impacts (~160 mPt) was the one configured with cultivation in wastewater followed directly by centrifugation (i.e., without flocculation neither electroflotation) and then drying.

In the study by Sandmann et al. (2021) the environmental impact of the tubular PBRs was lower than that of the mesh ultra-thin layer PBRs across all impact categories when using the FU of 1 kg of dry biomass. However, the ranking in the environmental performance of the two cultivation systems was inverted when considering the FU of 1 mmol of specific antioxidant capacity of the biomass, as the mesh ultra-thin layer PBRs had a 10-fold increased antioxidant capacity. For example, the mesh ultra-thin layer PBRs exhibited a GWP dropped to 41.61 kg $CO_{2\text{-eq}}$ $mmol^{-1}$ antioxidant capacity and a total endpoint impact of ~100 mPt, while the tubular PBRs had a GWP of 166.43 kg $CO_{2\text{-eq}}$ $mmol^{-1}$ antioxidant capacity and a total endpoint impact higher than 500 mPt. Therefore, the mesh ultra-thin layer PBR is a promising reactor configuration for sustainable production of bioactive substances.

6.2.2.Biofuels

Biofuels or their precursors from microalgae are the main products of several LCA studies, despite primary data at pilot scale are available only for the cultivation and harvesting stages, while downstream processes are modelled by data from the literature or extrapolated from laboratory scale.

LCI

The LCI reported by Passell et al. (2013) for their base case includes inputs in the upstream section per kg of dry biomass of 1.67 m^3 kg^{-1} for freshwater (salinity regulation and evaporation offset), 0.71 kg kg^{-1} for nutrients, 181 kg kg^{-1} for flue gas (~14% CO_2 content), and 85 kWh kg^{-1} for electricity (79% in the cultivation phase, 35% to supply paddlewheels). An output of 179 kg flue gas kg^{-1} biomass was also reported. In the downstream section, requirements per kg of extracted oil were 0.33 kg kg^{-1} for hexane, 2.21 kWh kg^{-1} for electricity (~90% in the belt filter press), and ~20.5 MJ kg^{-1} for heat, while data on transesterification and biodiesel combustion (simulated by GREET model) were not reported. The outputs in the oil extraction stage were 1.87 kg kg^{-1} for algae residue (oilcake), 0.67 kg kg^{-1} for low value lipids, and 17.35 L kg^{-1} for wastewater.

Gaber et al. (2021) reported the LCI for a pilot reactor (GWP-PBR system) with reference to the FU of 1 kg produced TFA. The data included cooling water by 105 tons kg^{-1}, cleaning water by 736 kg kg^{-1}, nutrients demand of 0.44 kg kg^{-1}, sodium hypochlorite by 0.83 kg kg^{-1}, total electricity consumption of ~280 kWh kg^{-1} (mainly due to the cultivation phase). Considering the TFA content of ~0.5 kg kg^{-1} dry biomass indicated by Passell et al. (2013), all these inputs have to be halved to obtain values comparable with the upstream LCI data by Passell et al. (2013). It follows that the consumptions of water, electricity, and nutrients by Gaber et al. (2021) are much higher, higher, and lower, respectively. This study estimated also two upscaled (and optimized) plants differing in the location. Their LCI exhibited markedly different data compared to the pilot, i.e., electricity consumption of ~22 kWh kg^{-1} (~7 kWh kg^{-1} in downstream processes) and no water for cooling. Moreover, the TFA extraction had a demand of MTBE solvent of 0.34 or 0.44 kg kg^{-1}. Therefore, this TFA lipid extraction via MTBE solvent was characterized by input amounts somehow similar to those of the extraction with hexane from Passell et al. (2013).

By referring to 1 kg biodiesel, Togarcheti et al. (2017) reported some LCI data, including 168 kWh kg^{-1} for energy consumption in pond cultivation (which dominated the total electricity consumption with 80% share, followed by mechanical dewatering), 170 MJ kg^{-1} for steam in spraydrying, and 4.33 kg kg^{-1} for $FeCl_3$ (flocculation). In the downstream section, lipid extraction accounted for hexane input of 6.22E-3 kg kg^{-1}, electrical energy of 12.67E-3 kWh kg^{-1}, and steam of 0.15 kg kg^{-1}, which are lower even by several orders of magnitude compared to the values reported in the other studies. Probably the values in the table were affected by some typos. Transesterification for conversion into biodiesel required methanol by 0.11 kg kg^{-1}.

With reference to 1 kg produced biodiesel, Branco-Vieira et al. (2020) reported ~585 m^3 kg^{-1} for freshwater (thermoregulation) and 13 m^3 kg^{-1} for seawater (culture medium), 1.7 kg kg^{-1} for nutrients, 24.06 kg kg^{-1} for CO_2, and 21.3 kWh kg^{-1} for electricity (95% in the centrifugation) in the upstream processes. Comparing these data with those of the pilot by Gaber et al. (2021) (assuming negligible effects due to the different FU), the consumption of materials (water and nutrients) was ~5 times higher, while the electricity consumption was ~13 times lower. The latter was significantly lower also compared to that by Togarcheti et al. (2017). In contrast, the upstream energy consumption was similar to that estimated by Gaber et al. (2021) for upscaled plants (~15 kWh kg^{-1}). In the LCI of downstream processes, Branco-Vieira et al. (2020) reported electrical energy consumption of 70.8 kWh kg^{-1} (40.79, 22.43, and 7.5 kWh kg^{-1} in paste freeze-drying, cell disruption, and lipid extraction, respectively), which is the highest among the LCA studies for microalgal biofuel reviewed in this section. However, this depends on the inclusion of the drying stage in the downstream processes. Other inputs were CO_2 (supercritical) for lipid extraction (9.57 kg kg^{-1}) and methanol for transesterification

(0.22 kg kg^{-1}, of which 0.1 kg kg^{-1} were recovered, thus the net input corresponds to that reported by Togarcheti et al. (2017)). Outputs included wastewater (1.36 m^3 kg^{-1}), nutrients loss (0.73 kg kg $^{-1}$), glycerol (0.1 kg kg^{-1}), and residual biomass (10.97 kg kg^{-1}).

LCIs from other studies are not directly comparable to those discussed so far. Indeed, Beal et al. (2015) reported the LCI (in the SI, Appendix G) of ten case studies by using 1 ha of facility area as functional unit. Ranges of inputs across the assessed scenarios included ~65 tons ha^{-1} for LDPE, ~30-450 tons ha^{-1} for fertilizers, 1.5-7.3 GWh ha^{-1} for electricity, 0.7-25 TJ ha^{-1} for heat, and 0-14.4 tons ha^{-1} for solvent. In Jez et al. (2017) the LCI data regarded only the upstream section, and were reported per 1 kg m^{-2} d^{-1} algae. However, it was not clear which area was considered (e.g., pond area, or different areas occupied by the specific process units).

LCIA

Some comparisons can be made on absolute values of environmental impacts evaluated by different studies. Freshwater eutrophication spanned across four order of magnitudes, from the lowest values of 6.6E-5 to 1.8E-4 kg$_{P\text{-}eq}$ kg^{-1} oil in Jez et al. (2017), through intermediate values of 3E-3 to 6E-3 kg$_{P\text{-}eq}$ kg^{-1} TFA in Gaber et al. (2021), up to the highest value of 6.4E-2 kg$_{P\text{-}eq}$ kg^{-1} biodiesel in Branco-Vieira et al. (2020). The study by Gaber et al. (2021) evaluated a GWP of 9.33-20.24 kg $CO_{2\text{-}eq}$ kg^{-1} TFA, which is quite lower than the range of ~70-250 kg $CO_{2\text{-}eq}$ kg^{-1} biodiesel by Togarcheti et al. (2017) and the value of 217 kg $CO_{2\text{-}eq}$ kg^{-1} biodiesel by Branco-Vieira et al. (2020), due to the assumptions by Gaber et al. (2021) of (i) a low electricity consumption for cultivation in the upscaled system simulated even in their baseline case and (ii) the use of biogenic CO_2 (from a biogas plant) not included as input flow in the LCI.

By using the FU of 1 MJ fuel combusted in CIDI vehicle, the assessment by Passell et al. (2013) showed that algae biodiesel was unsustainable compared to conventional diesel or soy biodiesel. For example, GWP and NER (energy in/energy out) were 1-2 orders of magnitude higher. This was caused by high impacts from cultivation and harvesting stages. By assessing a future case with improved algae biodiesel production (in terms of biomass productivity and algae-to-oil conversion, among other features), impacts and energy performance approached those of conventional (bio)diesel. In particular, base case and future case exhibited values of GWP of 2.88 vs. 0.18 kg$CO_{2\text{-}eq}$ MJ^{-1} and of NER of 33.44 vs. 1.37. A sensitivity analysis identified the case with 1.5 times increased algae oil/biomass ratio (0.75 kg kg^{-1}) as the only one leading to a favourable value of NER (i.e., < 1). However, it was still far from that of conventional diesel (0.64 vs. 0.18).

Beal et al. (2015) obtained promising results by assessing several case studies with output products including biocrude, animal feed, and ethanol. They used the FU of 1 ha of facility area for cultivation and processing, which was different from all other studies (Table 3). Results for energy performance showed EROI (energy out/energy in) values widely spanning from 0.34 to 8.35, thus prospecting promising opportunities. The highest value of EROI was estimated for a process scheme with HTL and wind power, which benefited of large biocrude yields, on-site electricity and heat production, low embedded energy inputs due to high levels of nutrient recycling, and renewable energy use. Regarding environmental impacts, almost all the case studies had neutral or beneficial impacts on ecosystem quality, climate change, and water depletion, while some cases were harmful for human health and non-renewable resources and others were beneficial for these categories. Fossil-derived electricity (mostly consumed for cultivation), animal feed substitution, and biocrude substitution were the most impactful parameters. The use of commercial nitrogen fertilizer played some role. A sensitivity analysis showed that the EROI values were significantly affected by the use of non-renewable energy rather than total energy the calculations, especially for case studies with wind power and large oil yields. Therefore, specifying which type of energy analysis is conducted (i.e., which EROI definition is used) is important to make comparisons across different studies. The CO_2 concentration (94% in the baseline, 10% in the less favorable case representing a typical power plant flue gas, which is more available) was the most influential parameter for the environmental impacts. Overall, different process pathways were promising (also for economics), but the requirements for high purity CO_2 and large amounts of fertilizer may be barriers to large-scale commercial deployment. Therefore, process optimization should be conducted with the use of atmospheric/waste carbon and waste nutrients.

Results from the environmental analysis by Jez et al. (2017) were not quite as optimistic, highlighting that microalgae were neither competitive yet with oil crops nor with fossil fuel. Large impacts were caused by the severe energy demand (electricity and heat) and material consumption for the algae biomass production. The use of photovoltaic energy reduced some impacts of microalgae oil compared to the base case of electricity from Italian grid (e.g., ~68% in climate change and 66% in fossil depletion), but it also increased some other impacts (human toxicity and metal depletion, due to heavy metals and chemical reagents necessary in PV panel production). Compared to fossil fuel oil and rapeseed oil, microalgae-derived oil with renewable energy led to lower impacts in three categories (marine eutrophication, freshwater ecotoxicity, and agricultural land occupation) over twelve. This was due to the destination of microalgae cake as substituted of soybean meal (avoided product), suggesting that coupling biofuel production with co-products valorization can be a promising strategy to achieve environmental sustainability.

Togarcheti et al. (2017) showed that cultivation in ORP with paddlewheel was the most energy intensive process, generating high impacts. The primary energy demand in cultivation was ~2500 MJ kg^{-1} biodiesel, and its reduction in the scenario with bioelectricity recovered from spent biomass derived biogas was negligible. This shows that the process is energetically unsustainable, as the energy content of microalgae biodiesel is ~40 MJ kg^{-1}. Harvesting and downstream processes accounted for 13% of the overall energy demand, which was a minor relative contribution, but it is important when compared with the biodiesel calorific value. Two additional scenarios were simulated by literature data, including improved biomass productivity (from 6 g m^{-2} d^{-1} in scenario 1 to 11.5 or 15 g m^{-2} d^{-1} in scenarios 2 or 3, respectively), air pumping for culture mixing (low energy demand, scenario 2), and U.S. average grid electricity (scenarios 2 and 3) instead of the hard coal Indian electricity of scenario 1. The additional scenarios had lower energy consumption in the cultivation phase (~18 and 122 kWh kg^{-1} biodiesel for scenario 2 and 3, respectively, against 168 kWh kg^{-1} biodiesel for scenario 1) and reduced the primary energy demand to ~200 MJ kg^{-1} or 1800 MJ kg^{-1}. Among the three scenarios, scenario 1 led to higher environmental impacts for all considered categories except one. The raceway pond was responsible of more than 80% contribution for all impact categories except one. Further scenarios were devised by increasing the productivity in scenario 1, showing a decrease in primary energy demand and in GWP up to ~60% (minimum values of ~700 MJ kg^{-1} and 70 kg $CO_{2\text{-eq}}$ kg^{-1}). From this study it can be concluded that the optimization of the cultivation stage in terms of energy consumption and biomass production is crucial for the process sustainability.

Branco-Vieira et al, (2020) found that PBR construction materials and energy consumption were the major contributors to the environmental impacts of microalgal biodiesel production. The relative contribution of the involved processes to each impact category is shown in Fig. 14. It shows that the upstream section (infrastructure, which was not included for downstream processes, plus cultivation and harvesting) caused more than 70% of the impact for most categories. Within the upstream group, cultivation operation is the most impactful item. The GWP was almost equally caused by the different processes, with a slight predominance of infrastructure, mostly due to the use of PMMA in the PBR construction. Cultivation was the second largest contributor to carbon emission due to the truck transportation of seawater. Carbon credits deriving from photosynthetic fixation of CO_2 produced a negative GWP (~–6%). Positive impacts (i.e., impacts with negative value) in other categories occurred due to protein-rich biomass and glycerol co-products considered as avoided products. Among other impact categories, it can be highlighted that ~90% of water consumption (WAC) was related to the cultivation step, due to the large amount of freshwater used for thermoregulation. In contrast, seawater for culture medium was used in a much lower amount and was even recycled by 90% after harvesting.

Within the downstream processes in Branco-Vieira et al, (2020), drying and cell disruption produced the largest impacts, followed by lipid extraction, while the effects of refining were almost negligible. Throughout the microalgal biodiesel production system, energy consumption was the highest contributor to the impacts on most of the analysed categories. The most energy-intensive step was biomass drying, requiring 43.7% of total energy use, i.e., 1.08 kWh MJ^{-1} biodiesel over a total of 2.47 kWh per MJ^{-1} biodiesel (which, by neglecting the energy content of co-products, would mean an energy out/energy in NER of only ~0.11).

The sensitivity analysis by Branco-Vieira et al, (2020) showed that energy consumption, water reuse and transportation distance of seawater from ocean were critical parameters affecting the overall environmental profile of the system. The fertilizer use had only small effects, probably due to the water reuse after harvesting. Overall, the study by Branco-Vieira et al, (2020) suggested that the reduction of environmental impacts can be pursued by using more sustainable materials for PBR construction, more renewable energy sources, and less-energy intensive drying technologies or processes for wet extraction of lipids. Moreover, the valorisation of co-products is an important aspect to further explore to improve the environmental sustainability of microalgal biodiesel production.

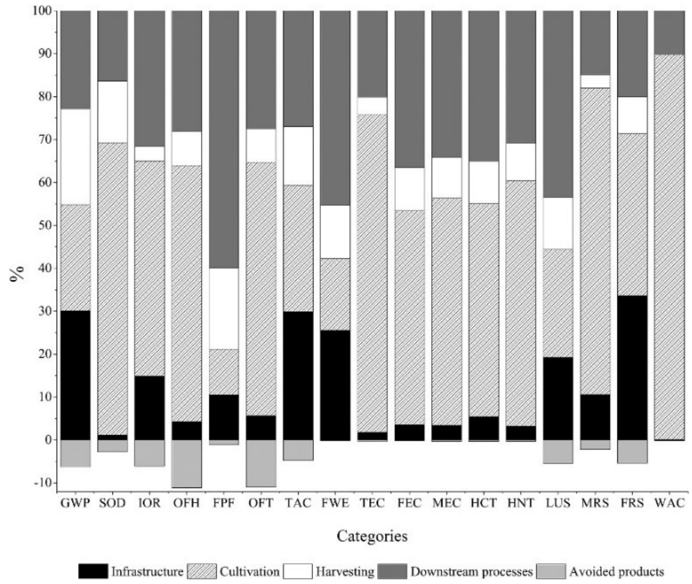


Fig. 14. Process contribution through different impact categories (ReCiPe method) in biodiesel production from *Phaeodactylum tricornutum* (FU of 1 MJ biodiesel) – Upstream: cultivation in bubble column PBRs, harvesting by centrifugation; Downstream: freeze-drying, cell disruption by ball mill, lipid extraction by supercritical CO_2, refining, and transesterification. GWP – global warming potential; SOD – stratospheric ozone depletion; IOR – ionizing radiation; OFH – ozone formation, human health; FPF – fine particulate matter formation; OFT – ozone formation, terrestrial ecosystems; TAC – terrestrial acidification; FWE – freshwater eutrophication; TEC – terrestrial ecotoxicity; FEC – freshwater ecotoxicity; MEC – marine ecotoxicity; HCT – human carcinogenic toxicity; HNT – human non-carcinogenic toxicity; LUS – land use; MRS – mineral resource scarcity; FRS – fossil resource scarcity; WAC – water consumption. Reproduced from Branco-Vieira et al. (2020), with permission from Elsevier, copyright 2020.

Gaber et al. (2021) analysed a process to produce TFA, which includes triacylglycerols (TAGs) applicable for biodiesel production. Their baseline scenario was devised with electricity consumption for upstream processes upscaled with 15% reduction from the techno-economic analysis by Tredici et al. (2016), resulting in an energy consumption of only 5.6% of that measured in the cultivation pilot. LCIA results are depicted in Fig. 15 for two Italian locations differing in productivity and yearly operating days (higher values for Sicily). Environmental impacts were around 15% less at the site with better growth conditions (see the numbers reported on the top of the bars in Fig. 15). Electricity consumption and infrastructure caused more than 50% of impact for almost all categories. Other operational materials (sodium hypochlorite, CO_2, solvents, operational oil) produced ~15-20% of impact, nutrients had an average contribution of ~10%, and tap water accounted for less than 10%.

Additionally, Gaber et al. (2021) simulated a *resource efficiency* scenario by including HTL treatment of residual biomass for nutrients recycle (63% N, and 90% P) and biocrude production (56% energy credit compared to the baseline). An *energy transition* scenario was simulated as the *resource efficiency* one with

Norwegian electricity mix, which is 98% hydropower. The scenario analysis showed that the *resource efficiency* process scheme led to a mild reduction of impacts (11% on average), while the *energy transition* was more effective in the mitigation of impacts (36% on average). In the latter scenario, infrastructure and other operational materials became the most impactful items. In a comparison with soybean oil (assessed by inventory database), the TFA production from microalgae was uncompetitive, as it led to higher impacts in all categories, except from land use. Overall, the LCA of Gaber et al. (2021) highlights that high productivity, nutrient recycling, bioenergy credits, and renewable energy can improve the environmental performance of microalgal biofuel production. However, it suffers from crucial barriers, such as the use of impactful infrastructure and operations.

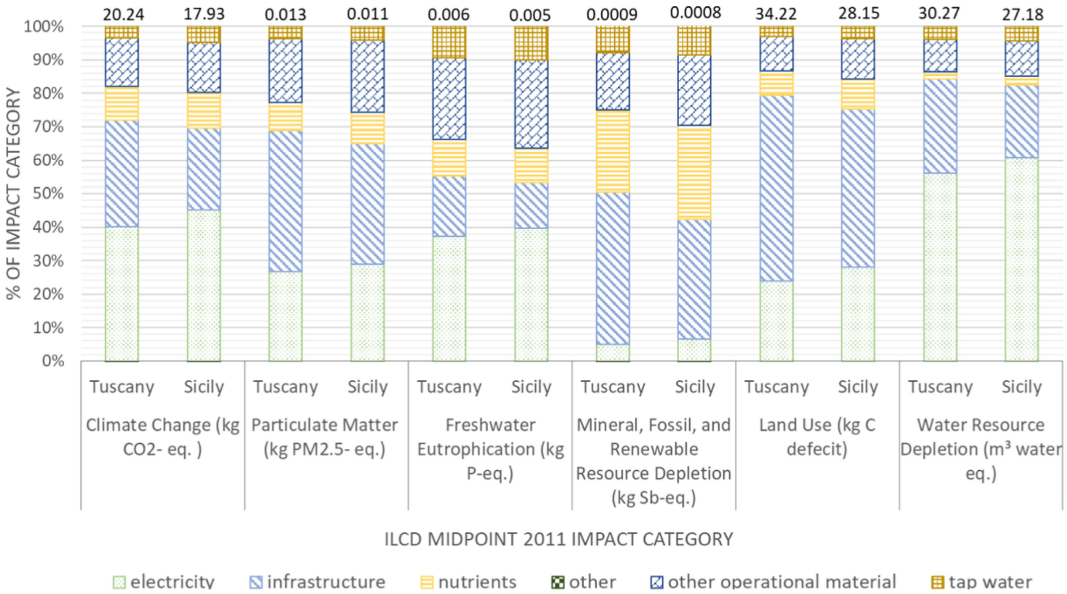


Fig. 15. Process contribution to environmental impact categories (ILCD Midpoint 2011 method) for the production of 1 kg TFA from *Nannochloropsis oceanica* – Upstream: cultivation in GWP-PBRs, harvesting by centrifugation; Downstream: cell disruption via homogenization, TFA extraction by solvent (MTBE), 3-phase separation, and solvent vaporization for recycle. Reproduced from Gaber et al. (2021).

6.2.3.High-value products

Three LCA studies based on pilot primary data were focused on high-value products as main products of the system. The LCA by Pérez-López et al. (2014b) assessed the production of several bioactive compounds for possible applications in the pharmaceutical and food/feed sectors, while the studies by Pérez-López et al. (2014a) and by Onorato and Rösch (2020) explored the production of astaxanthin, a red carotenoid pigment with antioxidant, photoprotection and provitamin A activity, which is widely used in the cosmetic, nutraceutical and feed sectors.

LCI

Pérez-López et al. (2014b) reported a detailed LCI referred to 1 kg of algal biomass. Inputs of the pilot for cultivation and extraction included ~0.82 kg kg^{-1} for construction materials (mainly stainless stell and methacrylate), 1296 L kg^{-1} for seawater, 129 L kg^{-1} for distilled water and tap water, ~510 g kg^{-1} for nutrients (mainly $NaNO_3$), 4.48 kg kg^{-1} for CO_2, 22.1 g kg^{-1} for hexane (quantities of other chemicals for extraction were not reported), and 68.7 kWh kg^{-1} for electricity from grid. Outputs were reported as well, including co-products (69.7 mg kg^{-1} for α-tocopherol, 3.76 g kg^{-1} for polyphenols, 3.21 g kg^{-1} for β-carotenoid, 8.76 g kg^{-1} for chlorophyll, 164 g kg^{-1} for lipids (45% PUFAs), 820 g kg^{-1} for residual algal paste), water emissions (1527 L kg^{-1} for wastewater, ~110 g kg^{-1} for nutrients, and roughly 0.6 kg kg^{-1} for chemicals), and solid waste to landfill or incineration (construction materials). The biogas production from residual biomass was characterized by relatively low flows, comprising recovered bioenergy (4.44 MJ kg^{-1}) and nutrients (~83 g kg^{-1}).

Onorato and Rösch (2020) reported inventory data on yearly basis, but the values with reference to 1 kg of biomass can be easily calculated (simply by dividing by the yearly biomass production in the red phase). The main inputs of cultivation in the Green Wall Panel (GWP), Flat Panel Airlift (FPA), and Unilayer Horizontal Tubular (UHT) PBRs can be summarized as 1779, 371, and 1691 L kg^{-1} for water, 127, 50, and 75 g kg^{-1} for nutrients, 57, 7, and 5 kg kg^{-1} for CO_2, and 312, 293, and 18 kWh kg^{-1} for electricity. The electricity required for harvesting was 6.0, 7.3 and 6.4 kWh kg^{-1} (mainly due to spraydrying) for GWP, FPA and UHT PBR, respectively. Finally, astaxanthin extraction with supercritical CO_2 solvent, which was simulated with literature data, was not inventoried.

In comparison with the LCIs of the three PBR systems from Onorato and Rösch (2020), the LCI of the small pilot assessed by Pérez-López et al. (2014b) required a quantity of water similar to that of the UHT PBR, an amount of nutrients four times that of the GWP PBR, a demand of CO_2 similar to that of the FPA and UHT PBRs, and an intermediate value of electrical energy. More in general, the LCI values reported above for microalgal systems operated to produce added-value products are quite aligned with those reported in section 6.2.1 for biomass production pilots, with the energy consumption of 18 kWh kg^{-1} of the UHT PBR pilot in Onorato and Rösch (2020) standing out as the lowest value.

Pérez-López et al. (2014a) used the FU of 800 g of astaxanthin (corresponding to the carotenoid mass extracted from a complete cycle of five cultures), thus their LCI results cannot be compared with those from other studies. However, for the sake of completeness, the main inputs are reported here: 800 g astaxanthin required ~13 kg construction materials (mainly stainless steel), 6795 kg water, 8 kg nutrients, 0.26 tons of CO_2, and 1980 kWh electricity (78% for lightning).

LCIA

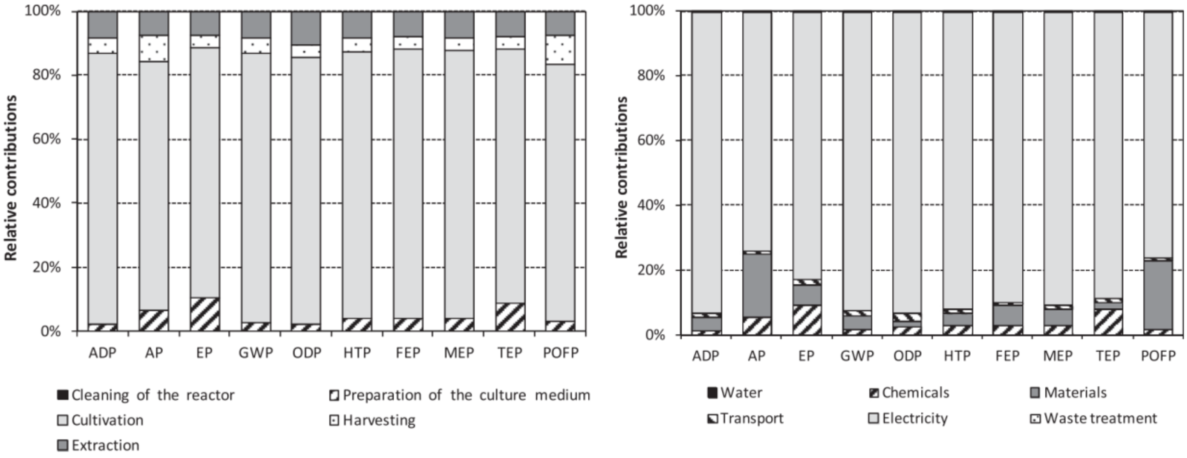
Two separate comparisons can be made on absolute values of environmental impacts from the three different studies, as they used different assessment methods, functional units, or system boundaries (Table 3 and Table 4).

(1) With reference to one kg of algal biomass, Pérez-López et al. (2014b) evaluated impacts for bioactive compounds production, by including acidification potential, GWP, and ozone layer depletion of 1.64 kg $SO_{2\text{-eq}}$ kg^{-1}, 56.7 kg $CO_{2\text{-eq}}$ kg^{-1}, and 5.67 mg CFC-11_{eq}, respectively; Onorato and Rösch (2020) estimated impacts for biomass production in four scenarios, i.e., GWP PBR with French grid, FPA PBR with either German or French grid, and UHT PBR with Portuguese grid, by including terrestrial acidification of 0.44, 0.52, 0.35, and 0.12 kg $SO_{2\text{-eq}}$ kg^{-1}, and climate change of 91.37, 265.21, 80.62, and 20.93 kg $CO_{2\text{-eq}}$ kg^{-1}, respectively, while ozone depletion was zero for all scenarios. Overall, the cultivation systems assessed by Onorato and Rösch (2020) tend to be less impactful across the different categories, but GHG emissions are reduced only in the case of the UHT PBR pilot.

(2) With reference to 1 kg astaxanthin, a value of GWP of 2325 kg $CO_{2\text{-eq}}$ kg^{-1} can be retrieved from Pérez-López et al. (2014a), which is intermediate compared to the values of climate change in the range 378-6119 kg $CO_{2\text{-eq}}$ kg^{-1} reported by Onorato and Rösch (2020). They evaluated environmental impacts across fifteen scenarios (with residual biomass use for biogas or feed production), obtaining the lowest value of climate change in the scenario of feed production (substitution of soybean), 10% astaxanthin content (the highest value considered) in the algal residue, and UHT PBR.

Pérez-López et al. (2014b) identified the culturing step for bioactive compounds production as the most impactful for all categories, with a relative contribution ranging from 73% to 97% depending on the impact category. Extraction was the second contributor, especially due to its electricity consumption. The electricity consumption for cultivation was significant (~58% of the total), but the impacts associated to this step were primarily caused by nutrients (especially nitrogen) and transport (of nutrients and seawater). Alternative nitrogen sources (inorganic and organic fertilizers, including recycled digestate and algal paste) were assessed in additional scenarios, showing an interesting potential for impact mitigation. Primary pilot data were integrated by literature data to assess a PBR-ORP hybrid system, showing a significant reduction of energy consumption (up to 56%) and of environmental burdens. However, cultivation in ORP may be affected by contamination issues, whose risk should be minimized in the production of high-value compounds.

In the production of astaxanthin (10% in the oleoresin product), Pérez-López et al. (2014a) compared the pilot with a lab-scale system, showing an efficient scale-up of the process that led to environmental impacts lower by 1-2 orders of magnitude. The cultivation stage was predominant in impacts generation, contributing by more than 80% in all impact categories (Fig. 16a). The second contribution to impacts was provided by the astaxanthin extraction step, followed by biomass harvesting. Electricity consumption, which occurred mainly for PBR lightning (78%), was the most impactful flow (more than 75% contribution in all impact categories), followed by materials and chemicals (Fig. 16b). In the scenario analysis, flat-panel PBRs (modelled by literature data) with artificial illumination provided the lowest impacts, which were reduced between 62% and 79% across the different categories. The study led to the conclusion that cultivation and electricity consumption were the main environmental hot spots to address for the implementation of industrial scale processes.

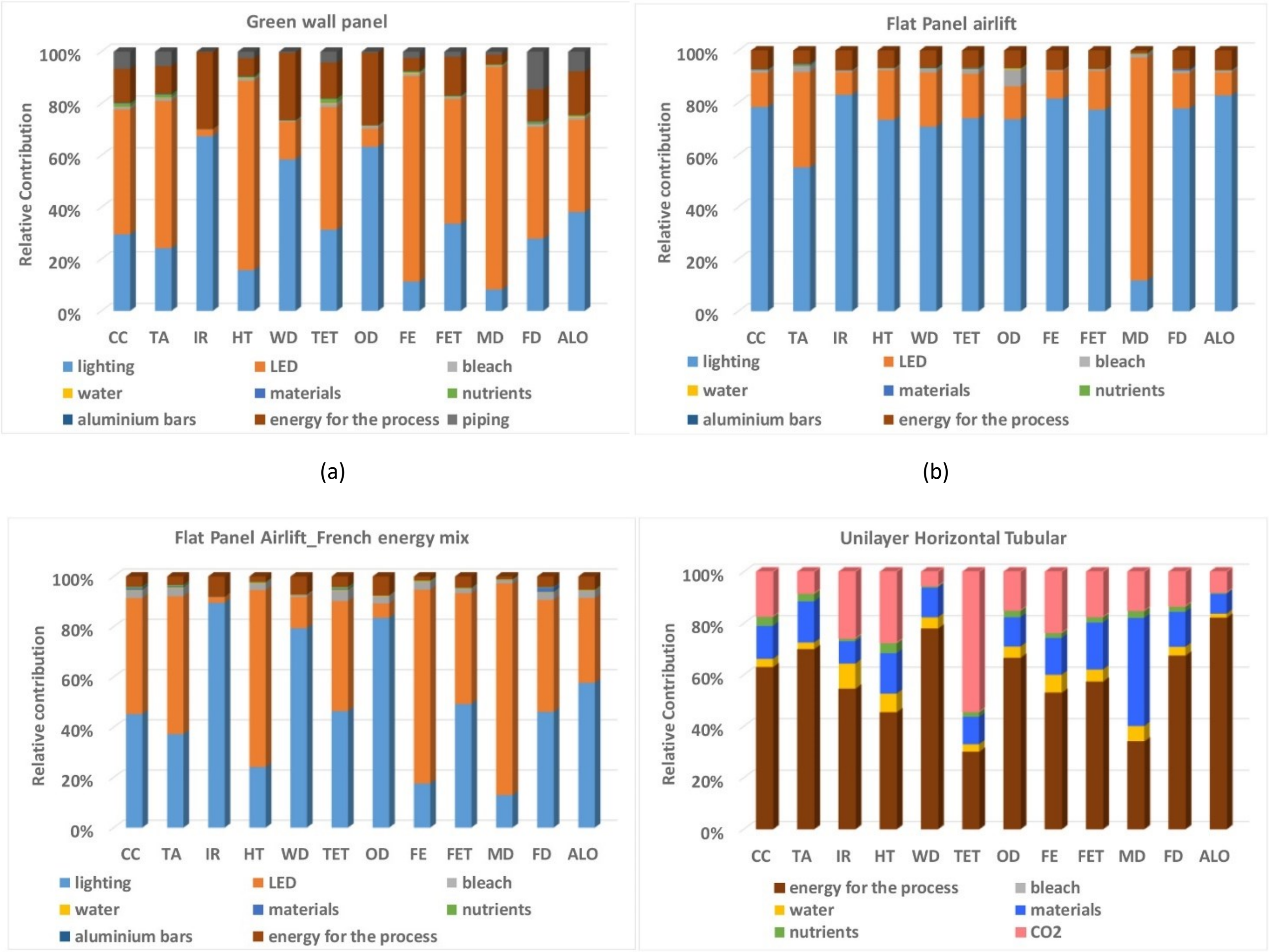


(a) (b)

Fig. 16. (a) Process contribution and (b) flow contribution to environmental impact categories (CML 2001 method) for the production of astaxanthin from *Haematococcus pluvialis* – Upstream: multi-stage cultivation in indoor airlift PBRs, settling, centrifugation, and spraydrying; Downstream: supercritical CO_2 extraction. Reproduced from Pérez-López et al. (2014a), with permission from Elsevier, copyright 2013.

With a similar process system, the extraction of astaxanthin (assumed to be pure) was studied also by Onorato and Rösch (2020), who compared different PBR configurations operated with either artificial or natural light (GWP or FPA PBR in the former case, UHT PBR in the latter case). In a first analysis, the LCA of the production of *H. pluvialis* (cultivation and harvesting) was carried out. In all systems, the second stage (red phase) of cultivation was the main hotspot for all the impact categories (contribution of 80–85%). An abstract of relative results is reported in Fig. 17 and Fig. 18, presenting the contributions of flows to the assessed environmental impacts and the environmental profile of the PBR systems, respectively. The GWP PBR exhibited LED production (electricity and materials) as the main hotspot, followed by electricity for lighting and then by energy for the process (including pumps and blower) (Fig. 17a). In the FPA PBR configuration with German energy mix, lighting was the main hotspot at the expense of reduced relative contributions of LED production and energy for the process (Fig. 17b). By switching the FPA PBR to the French energy mix (Fig. 17c), the relative contributions across the assessed impact categories were more similar to those of the GWP PBR, i.e., they were characterized by a predominant role of LED construction. For example, LED construction switched from less than 10% in the German grid case to ~50% in the French grid case. This behaviour was due to the lower impacts of lighting thanks to the electricity supply from a grid with a lower share of fossil fuel derived energy (9.1% in the French grid vs. 59.5% in the German grid). Overall, using artificial light had significant contributions to environmental impacts regardless the specific scenario of PBR configuration or grid mix considered. In contrast, the UHT PBR system, which did not use artificial light, exhibited the energy for the process as main hotspot, followed by CO_2 (production in liquid form) and materials (Fig. 17d).

Fig. 18 highlights the benefits of an electricity mix with a higher share of renewable energy (FPA with French mix compared to FPA with German grid) in reducing several environmental impacts. It also shows clearly that the UHT PBR system led to the lowest impacts across all categories, thus being the most promising for environmental-friendly production of algal biomass. Interestingly, this was even the configuration with the lowest productivity (Table 2) and with the most penalizing energy mix from the grid (89.2% fossil fuel). However, despite these drawbacks, it had the best environmental performance thanks to the lowest energy consumption allowed by the natural lighting (~6% of the energy consumption of GWP or FPA systems, see LCI data reported above).



(a) (b)

(c) (d)

Fig. 17. Flow contribution to environmental impact categories (ReCiPe method) for the production of *Haematococcus pluvialis* algal paste via multi-stage cultivation in different PBR systems: (a) GWP with French grid, (b) FPA with German grid, (c) FPA with French grid, or (d) UHT with Portuguese grid. The GWP and FPA PBRs were illuminated by LEDs, while the UHT PBR was illuminated by sunlight. The harvesting step included microfiltration, centrifugation, and spraydrying. CC – climate change; TA – terrestrial acidification; IR – ionising radiation; HT – human toxicity; WD – water depletion; TET – terrestrial ecotoxicity; OD – ozone depletion; FE – freshwater eutrophication; FET – freshwater ecotoxicity; MD – metal depletion; FD – fossil depletion; ALO – agricultural land occupation. Reproduced from Onorato and Rösch (2020), with permission from Elsevier, copyright 2020.

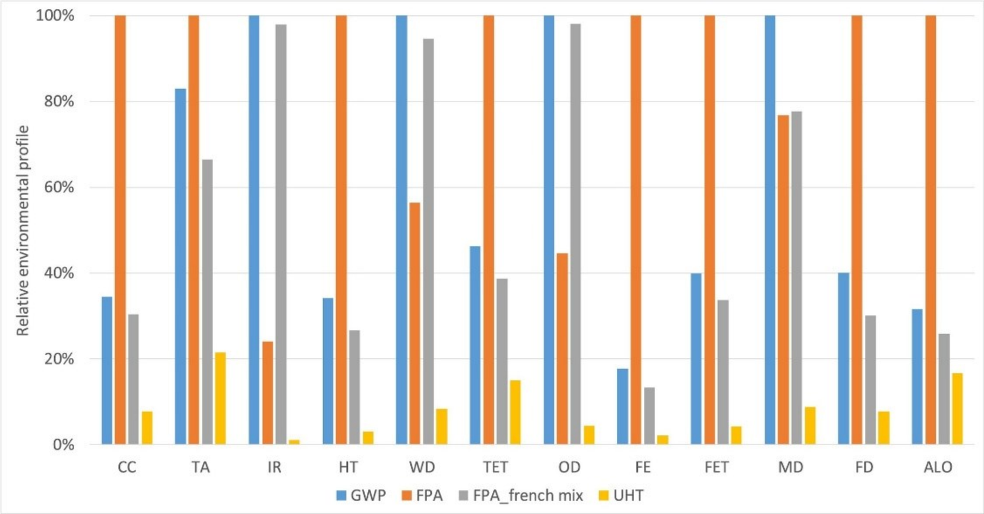


Fig. 18. Relative environmental profile (ReCiPe method) of different PBR systems for the production of *Haematococcus pluvialis* algal paste via multi-stage cultivation. The GWP and FPA PBRs were illuminated by LEDs, while the UHT PBR was illuminated by sunlight. The harvesting step included microfiltration, centrifugation, and spraydrying. CC – climate change; TA – terrestrial acidification; IR – ionising radiation; HT – human toxicity; WD – water depletion; TET – terrestrial ecotoxicity; OD – ozone depletion; FE – freshwater eutrophication; FET – freshwater ecotoxicity; MD – metal depletion; FD – fossil depletion; ALO – agricultural land occupation. Reproduced from Onorato and Rösch (2020), with permission from Elsevier, copyright 2020.

The second part of the study by Onorato and Rösch (2020) was devoted to the product system including the extraction of astaxanthin via supercritical CO_2, expanded with residual biomass use for either anaerobic digestion (electricity recovery) or feed production. The astaxanthin content in the biomass was low, i.e., 2.6%, 4.8% and 2.12% for the GWP, FPA, and UHT systems, respectively. Therefore, impacts for 1 kg astaxanthin FU were much higher than those for 1 kg biomass FU. In a scenario analysis, an enhanced value of 10% astaxanthin was included, showing that a higher carotenoid content can effectively reduce environmental impacts of the system expanded with anaerobic digestion; for example, climate change was reduced by 1/2-2/3, with the UHT PBR having the best performance in all scenarios. The system expanded with feed production (soybean substitution) showed a tendency to be less impactful, producing even negative impacts in two out of five categories, and reducing the other three (positive) impacts as the percent of astaxanthin in the biomass was let to increase. Among fifteen selected scenarios of different PBR configuration, grid mix, astaxanthin content and use of residual biomass, there was not a best solution for all impact categories; though still having good performance, the UHT PBR could be not identified as the best reactor configuration. The main conclusions from the comparative LCA by Onorato and Rösch (2020) were that use of renewable energy, installation in locations suitable for cultivation with sunlight, and valorisation of co-products can effectively improve the environmental sustainability of microalgal biomass production and astaxanthin extraction. Moreover, the maximization of the added-value compound productivity should be pursued in order to mitigate its high burdens. Further research efforts are needed to develop and characterize pilot PBR systems.

7. Conclusions and perspective

The present review was devoted to LCA studies with primary data from large microalgae-based production systems (pilot to industrial scale, >~100 L as culture volume). The search of relevant papers led to the selection of 14 studies satisfying the selection criteria. It was found that almost all the selected LCA studies were based on primary data only for the upstream processes (cultivation and harvesting), while downstream processes (if any) were simulated by literature data.

The adoption of different LCA methods (functional unit and LCIA method, among others) and of different modes of results presentation (aggregation and disaggregation of flows and processes in the impacts assessment, relative or absolute impacts) made a comprehensive comparison among studies impossible. Moreover, some lack of transparency (not available information) made elaboration of data difficult and interpretation of results ambiguous. However, the following conclusions can be drawn.

Cultivation and harvesting of microalgae in pilot plants showed an environmental performance affected significantly by electricity consumption and infrastructure. Outdoor systems illuminated by sunlight are by far more environmental-friendly than systems with artificial light. On the other hand, temperature regulation was an issue for energy consumption or water demand. Electricity grid mixes with high share of renewable sources were crucial to reduce environmental impacts. The geographic location can play a major role, which is inseparable from the choice of the reactor type. All the above is even more important by considering that, in the studies including downstream processes, the upstream section produced the largest impacts.

Several LCA studies on microalgal biofuels showed that they are not competitive with conventional (bio)fuels in terms of both net energy ratio and environmental impacts. However, some multi-product scenarios showed promising results. The production of high value biochemicals, e.g., antioxidants, was characterized by a short and poorly impactful downstream section and showed that the valorisation of co-products (residual biomass) was effective in reducing the environmental impacts.

Future studies should be aligned by a homogenization and harmonisation in the methods and should satisfy the essential requirement of clarity. More efforts should be devoted on the assessment of large-scale plants by using primary data. From the technological point of view, the cultivation stage should be optimized in terms of energy consumption and biomass production. The use of wastewater, waste CO_2 (flue gas), and alternative (or internally recycled) nutrients would boost the process sustainability. In a wider perspective including the downstream section, integrated biorefinery concepts deserve much more attention for the design and assessment of sustainable systems. In the case of high value compounds, the biochemical’s productivity should be maximized to mitigate the high environmental burdens.

Finally, the multi-dimensional character of sustainability can not be neglected. LCAs should be coupled with techno-economic analyses, with possible follow-up in enviro-economic optimization studies. This will allow the continuation of the journey along the roadmap towards the spread of commercial systems.

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Bibliography

Acién Fernández, F.G., Fernández Sevilla, J.M., Molina Grima, E., 2019. Costs analysis of microalgae production, in: Biofuels from Algae. Elsevier, pp. 551–566. https://doi.org/10.1016/B978-0-444-64192-2.00021-4

Ahmed, S.F., Mofijur, M., Nahrin, M., Chowdhury, S.N., Nuzhat, S., Alherek, M., Rafa, N., Ong, H.C., Nghiem, L.D., Mahlia, T.M.I., 2022. Biohydrogen production from wastewater-based microalgae: Progresses and challenges. Int. J. Hydrogen Energy 47, 37321–37342. https://doi.org/10.1016/j.ijhydene.2021.09.178

Alazaiza, M.Y.D., Albahnasawi, A., Ahmad, Z., Bashir, M.J.K., Al-Wahaibi, T., Abujazar, M.S.S., Abu Amr, S.S., Nassani, D.E., 2022. Potential use of algae for the bioremediation of different types of wastewater and contaminants: Production of bioproducts and biofuel for green circular economy. J. Environ. Manage. 324, 116415. https://doi.org/10.1016/j.jenvman.2022.116415

Amorim, M.L., Soares, J., Vieira, B.B., Leite, M. de O., Rocha, D.N., Aleixo, P.E., Falconí, J.H.H., Xavier Júnior, M. de L., Albino, L.F.T., Martins, M.A., 2021. Pilot-scale biorefining of Scenedesmus obliquus for the production of lipids and proteins. Sep. Purif. Technol. 270, 118775. https://doi.org/10.1016/j.seppur.2021.118775

Annevelink, B., Garcia Chavez, L., van Ree, R., Vural Gursel, I., 2022. Global biorefinery status report 2022.

Arena, R., Lima, S., Villanova, V., Moukri, N., Curcuraci, E., Messina, C., Santulli, A., Scargiali, F., 2021. Cultivation and biochemical characterization of isolated Sicilian microalgal species in salt and temperature stress conditions. Algal Res. 59, 102430. https://doi.org/10.1016/j.algal.2021.102430

Avila, R., Justo, Á., Carrero, E., Crivillés, E., Vicent, T., Blánquez, P., 2022. Water resource recovery coupling microalgae wastewater treatment and sludge co-digestion for bio-wastes valorisation at industrial pilot-scale. Bioresour. Technol. 343, 126080. https://doi.org/10.1016/j.biortech.2021.126080

Barreiro-Vescovo, S., Barbera, E., Bertucco, A., Sforza, E., 2020. Integration of Microalgae Cultivation in a Biogas Production Process from Organic Municipal Solid Waste: From Laboratory to Pilot Scale. ChemEngineering 4, 25. https://doi.org/10.3390/chemengineering4020025

Barros, A., Pereira, H., Campos, J., Marques, A., Varela, J., Silva, J., 2019. Heterotrophy as a tool to overcome the long and costly autotrophic scale-up process for large scale production of microalgae. Sci. Rep. 9, 13935. https://doi.org/10.1038/s41598-019-50206-z

Barsanti, L., Gualtieri, P., 2018. Is exploitation of microalgae economically and energetically sustainable? Algal Res. 31, 107–115. https://doi.org/10.1016/j.algal.2018.02.001

Beal, C.M., Gerber, L.N., Sills, D.L., Huntley, M.E., Machesky, S.C., Walsh, M.J., Tester, J.W., Archibald, I., Granados, J., Greene, C.H., 2015. Algal biofuel production for fuels and feed in a 100-ha facility: A comprehensive techno-economic analysis and life cycle assessment. Algal Res. 10, 266–279. https://doi.org/10.1016/j.algal.2015.04.017

Behera, B., Venkata Supraja, K., Paramasivan, B., 2021. Integrated microalgal biorefinery for the production and application of biostimulants in circular bioeconomy. Bioresour. Technol. 339, 125588. https://doi.org/10.1016/j.biortech.2021.125588

Bose, A., O'Shea, R., Lin, R., Long, A., Rajendran, K., Wall, D., De, S., Murphy, J.D., 2022. The marginal abatement cost of co-producing biomethane, food and biofertiliser in a circular economy system. Renew. Sustain. Energy Rev. 169, 112946. https://doi.org/10.1016/j.rser.2022.112946

Branco-Vieira, M., Costa, D.M.B., Mata, T.M., Martins, A.A., Freitas, M.A.V., Caetano, N.S., 2020. Environmental assessment of industrial production of microalgal biodiesel in central-south Chile. J. Clean. Prod. 266, 121756. https://doi.org/10.1016/j.jclepro.2020.121756

Branco-Vieira, M., Martin, S.S., Agurto, C., Dos Santos, M.A., Freitas, M.A.V., Mata, T.M., Martins, A.A., Caetano, N.S., 2018. Potential of Phaeodactylum tricornutum for biodiesel production under natural conditions in Chile. Energies 11. https://doi.org/10.3390/en11010054

Braun, J.C.A., Colla, L.M., 2022. Use of Microalgae for the Development of Biofertilizers and Biostimulants. BioEnergy Res. 1, 3. https://doi.org/10.1007/s12155-022-10456-8

Bussa, M., Eisen, A., Zollfrank, C., Röder, H., 2019. Life cycle assessment of microalgae products: State of the art and their potential for the production of polylactid acid. J. Clean. Prod. 213, 1299–1312. https://doi.org/10.1016/j.jclepro.2018.12.048

Carneiro, M.L.N.M., Pradelle, F., Braga, S.L., Gomes, M.S.P., Martins, A.R.F.A., Turkovics, F., Pradelle, R.N.C., 2017. Potential of biofuels from algae: Comparison with fossil fuels, ethanol and biodiesel in Europe and Brazil through life cycle assessment (LCA). Renew. Sustain. Energy Rev. 73, 632–653. https://doi.org/10.1016/j.rser.2017.01.152

Cavieres, L., Bazaes, J., Marticorena, P., Riveros, K., Medina, P., Sepúlveda, C., Riquelme, C., 2021. Pilot-scale phycoremediation using Muriellopsis sp. for wastewater reclamation in the Atacama Desert: microalgae biomass production and pigment recovery. Water Sci. Technol. 83, 331–343. https://doi.org/10.2166/wst.2020.576

Chen, P.H., Quinn, J.C., 2021. Microalgae to biofuels through hydrothermal liquefaction: Open-source techno-economic analysis and life cycle assessment. Appl. Energy 289, 116613. https://doi.org/10.1016/j.apenergy.2021.116613

Corrêa, P.S., Morais Júnior, W.G., Martins, A.A., Caetano, N.S., Mata, T.M., 2020. Microalgae Biomolecules: Extraction, Separation and Purification Methods. Processes 9, 10. https://doi.org/10.3390/pr9010010

de Jong, E., Higson, A., Walsh, P., Wellisch, M., 2012. Bio-based chemicals: Value added products from biorefineries.

de Souza, M.P., Hoeltz, M., Brittes Benitez, L., Machado, Ê.L., de Souza Schneider, R. de C., 2019. Microalgae and Clean Technologies: A Review. CLEAN – Soil, Air, Water 47, 1800380. https://doi.org/10.1002/clen.201800380

Dębowski, M., Świca, I., Kazimierowicz, J., Zieliński, M., 2022. Large Scale Microalgae Biofuel Technology—Development Perspectives in Light of the Barriers and Limitations. Energies 16, 81. https://doi.org/10.3390/en16010081

Devadas, V.V., Khoo, K.S., Chia, W.Y., Chew, K.W., Munawaroh, H.S.H., Lam, M.K., Lim, J.W., Ho, Y.C., Lee, K.T., Show, P.L., 2021. Algae biopolymer towards sustainable circular economy. Bioresour. Technol.

325, 124702. https://doi.org/10.1016/j.biortech.2021.124702

Di Lena, G., Casini, I., Lucarini, M., Lombardi-Boccia, G., 2019. Carotenoid profiling of five microalgae species from large-scale production. Food Res. Int. 120, 810–818. https://doi.org/10.1016/j.foodres.2018.11.043

Escobar, N., Laibach, N., 2021. Sustainability check for bio-based technologies: A review of process-based and life cycle approaches. Renew. Sustain. Energy Rev. 135, 110213. https://doi.org/10.1016/j.rser.2020.110213

Gaber, K., Rösch, C., Biondi, N., 2021. Life Cycle Assessment of Total Fatty Acid (TFA) Production from Microalgae Nannochloropsis oceanica at Different Sites and Under Different Sustainability Scenarios. Bioenergy Res. 1, 3. https://doi.org/10.1007/s12155-021-10279-z

Gerardo, M.L., Van Den Hende, S., Vervaeren, H., Coward, T., Skill, S.C., 2015. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. Algal Res. 11, 248–262. https://doi.org/10.1016/j.algal.2015.06.019

Goswami, R.K., Agrawal, K., Upadhyaya, H.M., Gupta, V.K., Verma, P., 2022. Microalgae conversion to alternative energy, operating environment and economic footprint: An influential approach towards energy conversion, and management. Energy Convers. Manag. 269. https://doi.org/10.1016/J.ENCONMAN.2022.116118

Gu, X., Martinez-Fernandez, J.S., Pang, N., Fu, X., Chen, S., 2020. Recent development of hydrothermal liquefaction for algal biorefinery. Renew. Sustain. Energy Rev. 121, 109707. https://doi.org/10.1016/j.rser.2020.109707

Haines, M., Vadlamani, A., Daniel Loty Richardson, W., Strous, M., 2022. Pilot-scale outdoor trial of a cyanobacterial consortium at pH 11 in a photobioreactor at high latitude. Bioresour. Technol. 354, 127173. https://doi.org/10.1016/j.biortech.2022.127173

Han, J., Thomsen, L., Pan, K., Wang, P., Wawilow, T., Osundeko, O., Wang, S., Theilen, U., Thomsen, C., 2020. Treating wastewater by indigenous microalgae strain in pilot platform located inside a municipal wastewater treatment plant. Environ. Technol. 41, 3261–3271. https://doi.org/10.1080/09593330.2019.1604816

Hauschild, M.Z., Rosenbaum, R.K., Olsen, S.I. (Eds.), 2018. Life Cycle Assessment. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-56475-3

He, S., Barati, B., Hu, X., Wang, S., 2023. Carbon migration of microalgae from cultivation towards biofuel production by hydrothermal technology: A review. Fuel Process. Technol. 240, 107563. https://doi.org/10.1016/j.fuproc.2022.107563

Hernández, D., Solana, M., Riaño, B., García-González, M.C., Bertucco, A., 2014. Biofuels from microalgae: Lipid extraction and methane production from the residual biomass in a biorefinery approach. Bioresour. Technol. 170, 370–378. https://doi.org/10.1016/j.biortech.2014.07.109

Huntley, M.E., Johnson, Z.I., Brown, S.L., Sills, D.L., Gerber, L., Archibald, I., Machesky, S.C., Granados, J., Beal, C., Greene, C.H., 2015. Demonstrated large-scale production of marine microalgae for fuels and feed. Algal Res. 10, 249–265. https://doi.org/10.1016/j.algal.2015.04.016

Iamtham, S., Sornchai, P., 2022. Biofixation of CO2 from a power plant through large-scale cultivation of Spirulina maxima. South African J. Bot. 147, 840–851. https://doi.org/10.1016/j.sajb.2022.03.028

ISO 14040:2006 - Environmental management — Life cycle assessment — Principles and framework, 2006. . Geneva.

ISO 14044:2006 - Environmental management — Life cycle assessment — Requirements and guidelines,

2006. . Geneva.

Jez, S., Spinelli, D., Fierro, A., Dibenedetto, A., Aresta, M., Busi, E., Basosi, R., 2017. Comparative life cycle assessment study on environmental impact of oil production from micro-algae and terrestrial oilseed crops. Bioresour. Technol. 239, 266–275. https://doi.org/10.1016/j.biortech.2017.05.027

Karpagam, R., Jawaharraj, K., Gnanam, R., 2021. Review on integrated biofuel production from microalgal biomass through the outset of transesterification route: a cascade approach for sustainable bioenergy. Sci. Total Environ. 766, 144236. https://doi.org/10.1016/j.scitotenv.2020.144236

Katiyar, R., Gurjar, B.R., Biswas, S., Pruthi, V., Kumar, N., Kumar, P., 2017. Microalgae: An emerging source of energy based bio-products and a solution for environmental issues. Renew. Sustain. Energy Rev. 72, 1083–1093. https://doi.org/10.1016/j.rser.2016.10.028

Katiyar, R., Gurjar, B.R., Kumar, A., Bharti, R.K., 2021. An integrated approach for phycoremediation of municipal wastewater and production of sustainable transportation fuel using oleaginous Chlorella sp. J. Water Process Eng. 42, 102183. https://doi.org/10.1016/j.jwpe.2021.102183

Ketzer, F., Skarka, J., Rösch, C., 2018. Critical Review of Microalgae LCA Studies for Bioenergy Production. Bioenergy Res. 11, 95–105. https://doi.org/10.1007/s12155-017-9880-1

Kim, J.Y., Jung, J.-M., Jung, S., Park, Y.-K., Tsang, Y.F., Lin, K.-Y.A., Choi, Y.-E., Kwon, E.E., 2022. Biodiesel from microalgae: Recent progress and key challenges. Prog. Energy Combust. Sci. 93, 101020. https://doi.org/10.1016/j.pecs.2022.101020

Koyande, A.K., Show, P.L., Guo, R., Tang, B., Ogino, C., Chang, J.S., 2019. Bio-processing of algal bio-refinery: a review on current advances and future perspectives. Bioengineered 10, 574–592. https://doi.org/10.1080/21655979.2019.1679697

Kumar, D., Singh, B., 2019. Algal biorefinery: An integrated approach for sustainable biodiesel production. Biomass and Bioenergy 131, 105398. https://doi.org/10.1016/j.biombioe.2019.105398

Lardon, L., Hélias, A., Sialve, B., Steyer, J.-P., Bernard, O., 2009. Life-Cycle Assessment of Biodiesel Production from Microalgae. Environ. Sci. Technol. 43, 6475–6481. https://doi.org/10.1021/es900705j

Laurens, L.M.L., Markham, J., Templeton, D.W., Christensen, E.D., Van Wychen, S., Vadelius, E.W., Chen-Glasser, M., Dong, T., Davis, R., Pienkos, P.T., 2017. Development of algae biorefinery concepts for biofuels and bioproducts; a perspective on process-compatible products and their impact on cost-reduction. Energy Environ. Sci. 10, 1716–1738. https://doi.org/10.1039/C7EE01306J

Lima, S., Lokesh, J., Schulze, P.S.C., Wijffels, R.H., Kiron, V., Scargiali, F., Petters, S., Bernstein, H.C., Morales-Sánchez, D., 2022. Flashing lights affect the photophysiology and expression of carotenoid and lipid synthesis genes in Nannochloropsis gaditana. J. Biotechnol. 360, 171–181. https://doi.org/10.1016/j.jbiotec.2022.11.012

Lima, S., Schulze, P.S.C., Schüler, L.M., Rautenberger, R., Morales-Sánchez, D., Santos, T.F., Pereira, H., Varela, J.C.S., Scargiali, F., Wijffels, R.H., Kiron, V., 2021. Flashing light emitting diodes (LEDs) induce proteins, polyunsaturated fatty acids and pigments in three microalgae. J. Biotechnol. 325, 15–24. https://doi.org/10.1016/j.jbiotec.2020.11.019

Lima, S., Villanova, V., Grisafi, F., Brucato, A., Scargiali, F., 2020. Combined effect of nutrient and flashing light frequency for a biochemical composition shift in Nannochloropsis gaditana grown in a quasi-isoactinic reactor. Can. J. Chem. Eng. 98, 1944–1954. https://doi.org/10.1002/cjce.23776

Liyanaarachchi, V.C., Premaratne, M., Ariyadasa, T.U., Nimarshana, P.H.V., Malik, A., 2021. Two-stage cultivation of microalgae for production of high-value compounds and biofuels: A review. Algal Res. 57, 102353. https://doi.org/10.1016/j.algal.2021.102353

Long, J., Jia, J., Gong, Y., Han, D., Hu, Q., 2022. Assessment of Eicosapentaenoic Acid (EPA) Production from Filamentous Microalga Tribonema aequale: From Laboratory to Pilot-Scale Study. Mar. Drugs 20, 343. https://doi.org/10.3390/md20060343

Markou, G., Ilkiv, B., Brulé, M., Antonopoulos, D., Chakalis, L., Arapoglou, D., Chatzipavlidis, I., 2022. Methane production through anaerobic digestion of residual microalgal biomass after the extraction of valuable compounds. Biomass Convers. Biorefinery 12, 419–426. https://doi.org/10.1007/s13399-020-00703-3

Masojídek, J., Gómez-Serrano, C., Ranglová, K., Cicchi, B., Encinas Bogeat, Á., Câmara Manoel, J.A., Sanches Zurano, A., Silva Benavides, A.M., Barceló-Villalobos, M., Robles Carnero, V.A., Ördög, V., Gómez Pinchetti, J.L., Vörös, L., Arbib, Z., Rogalla, F., Torzillo, G., Lopez Figueroa, F., Acién-Fernándéz, F.G., 2022. Photosynthesis Monitoring in Microalgae Cultures Grown on Municipal Wastewater as a Nutrient Source in Large-Scale Outdoor Bioreactors. Biology (Basel). 11, 1380. https://doi.org/10.3390/biology11101380

Merlo, S., Gabarrell Durany, X., Pedroso Tonon, A., Rossi, S., 2021. Marine Microalgae Contribution to Sustainable Development. Water 13, 1373. https://doi.org/10.3390/w13101373

Mishra, S., Roy, M., Mohanty, K., 2019. Microalgal bioenergy production under zero-waste biorefinery approach: Recent advances and future perspectives. Bioresour. Technol. 292, 122008. https://doi.org/10.1016/j.biortech.2019.122008

Mohan, N., Rao, P.H., Boopathy, A.B., Rengasamy, R., Chinnasamy, S., 2021. A sustainable process train for a marine microalga-mediated biomass production and CO2 capture: A pilot-scale cultivation of Nannochloropsis salina in open raceway ponds and harvesting through electropreciflocculation. Renew. Energy 173, 263–272. https://doi.org/10.1016/j.renene.2021.03.147

Montalvo, G.E.B., Thomaz-Soccol, V., Vandenberghe, L.P.S., Carvalho, J.C., Faulds, C.B., Bertrand, E., Prado, M.R.M., Bonatto, S.J.R., Soccol, C.R., 2019. Arthrospira maxima OF15 biomass cultivation at laboratory and pilot scale from sugarcane vinasse for potential biological new peptides production. Bioresour. Technol. 273, 103–113. https://doi.org/10.1016/j.biortech.2018.10.081

Morillas-España, A., Lafarga, T., Sánchez-Zurano, A., Acién-Fernández, F.G., Rodríguez-Miranda, E., Gómez-Serrano, C., González-López, C.V., 2021. Year-long evaluation of microalgae production in wastewater using pilot-scale raceway photobioreactors: Assessment of biomass productivity and nutrient recovery capacity. Algal Res. 60, 102500. https://doi.org/10.1016/j.algal.2021.102500

Morya, R., Raj, T., Lee, Y., Kumar Pandey, A., Kumar, D., Rani Singhania, R., Singh, S., Prakash Verma, J., Kim, S.-H., 2022. Recent updates in biohydrogen production strategies and life–cycle assessment for sustainable future. Bioresour. Technol. 366, 128159. https://doi.org/10.1016/j.biortech.2022.128159

Nagarajan, D., Dong, C., Chen, C., Lee, D., Chang, J., 2021. Biohydrogen production from microalgae—Major bottlenecks and future research perspectives. Biotechnol. J. 16, 2000124. https://doi.org/10.1002/biot.202000124

Nanda, N., Bharadvaja, N., 2022. Algal bioplastics: current market trends and technical aspects. Clean Technol. Environ. Policy 24, 2659–2679. https://doi.org/10.1007/s10098-022-02353-7

Olguín, E.J., Sánchez-Galván, G., Arias-Olguín, I.I., Melo, F.J., González-Portela, R.E., Cruz, L., De Philippis, R., Adessi, A., 2022. Microalgae-Based Biorefineries: Challenges and Future Trends to Produce Carbohydrate Enriched Biomass, High-Added Value Products and Bioactive Compounds. Biology (Basel). 11, 1146. https://doi.org/10.3390/biology11081146

Oncel, S.S., 2015. Biohydrogen from Microalgae, Uniting Energy, Life, and Green Future, in: Handbook of Marine Microalgae. Elsevier, pp. 159–196. https://doi.org/10.1016/B978-0-12-800776-1.00011-X

Onorato, C., Rösch, C., 2020. Comparative life cycle assessment of astaxanthin production with

Haematococcus pluvialis in different photobioreactor technologies. Algal Res. 50, 102005. https://doi.org/10.1016/j.algal.2020.102005

Parsons, S., Allen, M.J., Chuck, C.J., 2020. Coproducts of algae and yeast-derived single cell oils: A critical review of their role in improving biorefinery sustainability. Bioresour. Technol. 303, 122862. https://doi.org/10.1016/j.biortech.2020.122862

Passell, H., Dhaliwal, H., Reno, M., Wu, B., Ben Amotz, A., Ivry, E., Gay, M., Czartoski, T., Laurin, L., Ayer, N., 2013. Algae biodiesel life cycle assessment using current commercial data. J. Environ. Manage. 129, 103–111. https://doi.org/10.1016/j.jenvman.2013.06.055

Pérez-López, P., de Vree, J.H., Feijoo, G., Bosma, R., Barbosa, M.J., Moreira, M.T., Wijffels, R.H., van Boxtel, A.J.B., Kleinegris, D.M.M., 2017. Comparative life cycle assessment of real pilot reactors for microalgae cultivation in different seasons. Appl. Energy 205, 1151–1164. https://doi.org/10.1016/j.apenergy.2017.08.102

Pérez-López, P., González-García, S., Jeffryes, C., Agathos, S.N., McHugh, E., Walsh, D., Murray, P., Moane, S., Feijoo, G., Moreira, M.T., 2014a. Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. J. Clean. Prod. 64, 332–344. https://doi.org/10.1016/j.jclepro.2013.07.011

Pérez-López, P., González-García, S., Ulloa, R.G., Sineiro, J., Feijoo, G., Moreira, M.T., 2014b. Life cycle assessment of the production of bioactive compounds from Tetraselmis suecica at pilot scale. J. Clean. Prod. 64, 323–331. https://doi.org/10.1016/j.jclepro.2013.07.028

Plouviez, M., Chambonnière, P., Shilton, A., Packer, M.A., Guieysse, B., 2019. Nitrous oxide (N2O) emissions during real domestic wastewater treatment in an outdoor pilot-scale high rate algae pond. Algal Res. 44, 101670. https://doi.org/10.1016/j.algal.2019.101670

Premaratne, M., Nishshanka, G.K.S.H., Anthonio, R.A.D.P., Liyanaarachchi, V.C., Thevarajah, B., Nimarshana, P.H.V., Malik, A., Ariyadasa, T.U., 2022. Resource recovery from waste streams for production of microalgae biomass: A sustainable approach towards high-value biorefineries. Bioresour. Technol. Reports 18, 101070. https://doi.org/10.1016/j.biteb.2022.101070

Rajesh Banu, J., Preethi, Kavitha, S., Gunasekaran, M., Kumar, G., 2020. Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis. Bioresour. Technol. 302, 122822. https://doi.org/10.1016/j.biortech.2020.122822

Rentería-Mexía, A., Ulloa-Mercado, G., Gortáres-Moroyoqui, P., González-Mercado, A., Sánchez, M., Sineiro, J., Núñez, M.J., 2022. Antioxidant potential and antiangiogenic activity of Tetraselmis suecica grown in a semicontinuous culture. J. Chem. Technol. Biotechnol. 97, 2528–2536. https://doi.org/10.1002/jctb.7113

Richmond, A., Hu, Q., 2013. Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition, Handbook of Microalgal Culture: Applied Phycology and Biotechnology: Second Edition. Wiley, Oxford, UK. https://doi.org/10.1002/9781118567166

Roy, M., Mohanty, K., 2019. A comprehensive review on microalgal harvesting strategies: Current status and future prospects. Algal Res. 44, 101683. https://doi.org/10.1016/j.algal.2019.101683

Sánchez Zurano, A., Garrido Cárdenas, J.A., Gómez Serrano, C., Morales Amaral, M., Acién-Fernández, F.G., Fernández Sevilla, J.M., Molina Grima, E., 2020. Year-long assessment of a pilot-scale thin-layer reactor for microalgae wastewater treatment. Variation in the microalgae-bacteria consortium and the impact of environmental conditions. Algal Res. 50, 101983. https://doi.org/10.1016/j.algal.2020.101983

Sandmann, M., Smetana, S., Heinz, V., Rohn, S., 2021. Comparative life cycle assessment of a mesh ultra-thin layer photobioreactor and a tubular glass photobioreactor for the production of bioactive algae extracts. Bioresour. Technol. 340, 125657. https://doi.org/10.1016/j.biortech.2021.125657

Schade, S., Meier, T., 2019. A comparative analysis of the environmental impacts of cultivating microalgae in different production systems and climatic zones: A systematic review and meta-analysis. Algal Res. 40, 101485. https://doi.org/10.1016/j.algal.2019.101485

Schaubroeck, T., Schaubroeck, S., Heijungs, R., Zamagni, A., Brandão, M., Benetto, E., 2021. Attributional & Consequential Life Cycle Assessment: Definitions, Conceptual Characteristics and Modelling Restrictions. Sustainability 13, 7386. https://doi.org/10.3390/su13137386

Schneider, R. de C. de S., de Moura Lima, M., Hoeltz, M., de Farias Neves, F., John, D.K., de Azevedo, A., 2018. Life cycle assessment of microalgae production in a raceway pond with alternative culture media. Algal Res. 32, 280–292. https://doi.org/10.1016/j.algal.2018.04.012

Sen, S., Sarkar, S., Bhowmick, T.K., Gayen, K., 2022. The choice of algae strain for the biofuel production: Native, genetically modified, and microbial consortia, in: 3rd Generation Biofuels. Elsevier, pp. 3–32. https://doi.org/10.1016/B978-0-323-90971-6.00036-X

Sevigné Itoiz, E., Fuentes-Grünewald, C., Gasol, C.M., Garcés, E., Alacid, E., Rossi, S., Rieradevall, J., 2012. Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant. Biomass and Bioenergy 39, 324–335. https://doi.org/10.1016/j.biombioe.2012.01.026

Shah, M.M.R., Liang, Y., Cheng, J.J., Daroch, M., 2016. Astaxanthin-Producing Green Microalga Haematococcus pluvialis: From Single Cell to High Value Commercial Products. Front. Plant Sci. 7, 531. https://doi.org/10.3389/fpls.2016.00531

Show, K.Y., Lee, D.J., Tay, J.H., Lee, T.M., Chang, J.S., 2015. Microalgal drying and cell disruption - Recent advances. Bioresour. Technol. 184, 258–266. https://doi.org/10.1016/J.BIORTECH.2014.10.139

Silva, A.G., Carter, R., Merss, F.L.M., Corrêa, D.O., Vargas, J.V.C., Mariano, A.B., Ordonez, J.C., Scherer, M.D., 2015. Life cycle assessment of biomass production in microalgae compact photobioreactors. GCB Bioenergy 7, 184–194. https://doi.org/10.1111/gcbb.12120

Silva, S.C., Ferreira, I.C.F.R., Dias, M.M., Barreiro, M.F., 2020. Microalgae-Derived Pigments: A 10-Year Bibliometric Review and Industry and Market Trend Analysis. Molecules 25, 3406. https://doi.org/10.3390/molecules25153406

Singh, G., Patidar, S.K., 2018. Microalgae harvesting techniques: A review. J. Environ. Manage. 217, 499–508. https://doi.org/10.1016/J.JENVMAN.2018.04.010

Slade, R., Bauen, A., 2013. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass and Bioenergy 53, 29–38. https://doi.org/10.1016/j.biombioe.2012.12.019

Solovchenko, A.E., Khozin-Goldberg, I., Didi-Cohen, S., Cohen, Z., Merzlyak, M.N., 2008. Effects of light and nitrogen starvation on the content and composition of carotenoids of the green microalga Parietochloris incisa. Russ. J. Plant Physiol. 55, 455–462. https://doi.org/10.1134/S1021443708040043

Stephenson, A.L., Kazamia, E., Dennis, J.S., Howe, C.J., Scott, S.A., Smith, A.G., 2010. Life-Cycle Assessment of Potential Algal Biodiesel Production in the United Kingdom: A Comparison of Raceways and Air-Lift Tubular Bioreactors. Energy & Fuels 24, 4062–4077. https://doi.org/10.1021/ef1003123

Sung, Y.J., Lee, J.S., Yoon, H.K., Ko, H., Sim, S.J., 2021. Outdoor cultivation of microalgae in a coal-fired power plant for conversion of flue gas CO2 into microalgal direct combustion fuels. Syst. Microbiol. Biomanufacturing 1, 90–99. https://doi.org/10.1007/s43393-020-00007-7

Tejido-Nuñez, Y., Aymerich, E., Sancho, L., Refardt, D., 2020. Co-cultivation of microalgae in aquaculture water: Interactions, growth and nutrient removal efficiency at laboratory- and pilot-scale. Algal Res. 49, 101940. https://doi.org/10.1016/j.algal.2020.101940

Togarcheti, S.C., Mediboyina, M. kumar, Chauhan, V.S., Mukherji, S., Ravi, S., Mudliar, S.N., 2017. Life cycle assessment of microalgae based biodiesel production to evaluate the impact of biomass productivity and energy source. Resour. Conserv. Recycl. 122, 286–294. https://doi.org/10.1016/j.resconrec.2017.01.008

Tredici, M.R., Rodolfi, L., Biondi, N., Bassi, N., Sampietro, G., 2016. Techno-economic analysis of microalgal biomass production in a 1-ha Green Wall Panel (GWP®) plant. Algal Res. 19, 253–263. https://doi.org/10.1016/j.algal.2016.09.005

Ubando, A.T., Anderson S. Ng, E., Chen, W.H., Culaba, A.B., Kwon, E.E., 2022. Life cycle assessment of microalgal biorefinery: A state-of-the-art review. Bioresour. Technol. 360, 127615. https://doi.org/10.1016/J.BIORTECH.2022.127615

Ubando, A.T., Rivera, D.R.T., Chen, W.-H., Culaba, A.B., 2019. A comprehensive review of life cycle assessment (LCA) of microalgal and lignocellulosic bioenergy products from thermochemical processes. Bioresour. Technol. 291, 121837. https://doi.org/10.1016/j.biortech.2019.121837

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. J. Renew. Sustain. Energy 2, 012701. https://doi.org/10.1063/1.3294480

Usai, A., Theodoropoulos, C., Di Caprio, F., Altimari, P., Cao, G., Concas, A., 2023. Structured population balances to support microalgae-based processes: Review of the state-of-art and perspectives analysis. Comput. Struct. Biotechnol. J. 21, 1169–1188. https://doi.org/10.1016/j.csbj.2023.01.042

Venkata Mohan, S., Hemalatha, M., Chakraborty, D., Chatterjee, S., Ranadheer, P., Kona, R., 2020. Algal biorefinery models with self-sustainable closed loop approach: Trends and prospective for blue-bioeconomy. Bioresour. Technol. 295, 122128. https://doi.org/10.1016/j.biortech.2019.122128

Venkata Subhash, G., Rajvanshi, M., Raja Krishna Kumar, G., Shankar Sagaram, U., Prasad, V., Govindachary, S., Dasgupta, S., 2022. Challenges in microalgal biofuel production: A perspective on techno economic feasibility under biorefinery stratagem. Bioresour. Technol. 343, 126155. https://doi.org/10.1016/j.biortech.2021.126155

Villanova, V., Galasso, C., Fiorini, F., Lima, S., Brönstrup, M., Sansone, C., Brunet, C., Brucato, A., Scargiali, F., 2021. Biological and chemical characterization of new isolated halophilic microorganisms from saltern ponds of Trapani, Sicily. Algal Res. 54. https://doi.org/10.1016/j.algal.2021.102192

Villaró, S., Sánchez-Zurano, A., Ciardi, M., Alarcón, F.J., Clagnan, E., Adani, F., Morillas-España, A., Álvarez, C., Lafarga, T., 2022. Production of microalgae using pilot-scale thin-layer cascade photobioreactors: Effect of water type on biomass composition. Biomass and Bioenergy 163, 106534. https://doi.org/10.1016/j.biombioe.2022.106534

Wu, W., Chang, J.-S., 2019. Integrated algal biorefineries from process systems engineering aspects: A review. Bioresour. Technol. 291, 121939. https://doi.org/10.1016/j.biortech.2019.121939

Xu, L., Weathers, P.J., Xiong, X.R., Liu, C.Z., 2009. Microalgal bioreactors: Challenges and opportunities. Eng. Life Sci. 9, 178–189. https://doi.org/10.1002/elsc.200800111

Yadav, K., Vasistha, S., Nawkarkar, P., Kumar, S., Rai, M.P., 2022. Algal biorefinery culminating multiple value-added products: recent advances, emerging trends, opportunities, and challenges. 3 Biotech 12, 244. https://doi.org/10.1007/s13205-022-03288-y

Yun, J.-H., Cho, D.-H., Heo, J., Lee, Y.J., Lee, B., Chang, Y.K., Kim, H.-S., 2019. Evaluation of the potential of Chlorella sp. HS2, an algal isolate from a tidal rock pool, as an industrial algal crop under a wide range of abiotic conditions. J. Appl. Phycol. 31, 2245–2258. https://doi.org/10.1007/s10811-019-1751-z

Zabed, H.M., Akter, S., Yun, J., Zhang, G., Zhang, Y., Qi, X., 2020. Biogas from microalgae: Technologies, challenges and opportunities. Renew. Sustain. Energy Rev. 117, 109503. https://doi.org/10.1016/j.rser.2019.109503

Zedler, J.A.Z., Gangl, D., Guerra, T., Santos, E., Verdelho, V. V., Robinson, C., 2016. Pilot-scale cultivation of wall-deficient transgenic Chlamydomonas reinhardtii strains expressing recombinant proteins in the chloroplast. Appl. Microbiol. Biotechnol. 100, 7061–7070. https://doi.org/10.1007/s00253-016-7430-y

Zheng, H., Wang, Y., Li, S., Nagarajan, D., Varjani, S., Lee, D.-J., Chang, J.-S., 2022. Recent advances in lutein production from microalgae. Renew. Sustain. Energy Rev. 153, 111795. https://doi.org/10.1016/j.rser.2021.111795