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Microalgae production in an industrial-scale photobioreactors plant: A comprehensive Life Cycle assessment

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ABSTRACT

Microalgae cultivation provides multiple opportunities to produce valuable bioproducts, but greater clarity must be achieved regarding the real sustainability of current technologies. Numerous life cycle assessment (LCA) studies have been conducted so far. However, most of them were based on literature data and/or extrapolations of lab-scale results, while only a few studies used primary data from pilot or full-scale microalgal plants. Moreover, the obtained results showed great variability, leaving the debate on microalgae sustainability fully open.

This work presents a thorough LCA based on primary data from an industrial-scale microalgal facility located in Caltagirone, Italy. The plant is based on vertically-stacked horizontal photobioreactors (total volume of 40.4 m^3) installed in a greenhouse and has a capacity of 1200 kg_{DW}/y (*Chlorella vulgaris*). A cradle-to-gate assessment was performed with the functional unit of 1 kg_{DW} biomass, including operational and infrastructural data. The results emphasized the key role in the generation of potential impacts played by cultivation among process stages and by chemicals (nutrients and cleaning agents) and electricity (mainly for agitation and thermoregulation) among flow types. In comparison with studies from the literature, the analysed microalgal plant has an intermediate environmental performance (e.g., global warming potential of 153 kg $CO_{2,eq}/kg_{DW}$). This result is encouraging, as it comes from a reliable assessment built on full-scale primary data. On the other hand, it highlights the need to explore alternative strategies (e.g., industrial symbiosis and circular bioeconomy) to reduce the environmental footprint of the process and enhance its economic attractiveness.

1. Introduction

The microalgae production sector is rapidly expanding. These organisms have a great potential for various commercial applications, including the production of biofuels/bioenergy (Choi et al., 2019; Moshood et al., 2021), biochemicals (Russell et al., 2022), and biomaterials (Mastropetros et al., 2022). Additionally, microalgae cultivation can support climate change mitigation by CO_2 photosynthetic capture and conversion (Daneshvar et al., 2022; Hou et al., 2024; Sawayama et al., 1999; Wang et al., 2008, 2023). The

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biorefinery approach has emerged recently from a perspective of limiting waste and maximising environmental and economic performances (Chew et al., 2017; Okeke et al., 2022), and promising developments have been presented. For example, microalgae production can be coupled to wastewater treatment with nutrient recovery, thereby enhancing circular bioeconomy practices (Avila et al., 2022; Herrera et al., 2021; Ortiz Tena et al., 2024). However, a great deal of research is still needed to improve the economic and environmental sustainability of microalgae-based production systems, which is crucial to ensure the spread of competitive industrial applications and to increase the market share. Indeed, techno-economic feasibility (Yadav et al., 2022) and environmental friendliness (Ubando et al., 2022) are still challenging and must overcome several bottlenecks (Dębowski et al., 2022).

As far as environmental sustainability is concerned, the Life Cycle Assessment (LCA) methodology is the most frequently used approach for the evaluation of bio-based technologies (Escobar and Laibach, 2021). Specifically, the LCA application has been well-documented in the literature for numerous microalgae-based products (Rajesh Banu et al., 2020; Ubando et al., 2022; Wang et al., 2023), particularly for food and feeds (Smetana et al., 2017), biofuels (Mu et al., 2020), and higher-value commercial products - such as astaxanthin – all of which involve downstream processes (Pérez-López et al., 2014a; Zlaugotne et al., 2023). According to Bradley et al. (2023), biofuels derived from microalgae have not yet reached the maturity necessary to compete with petroleum-derived fuels from the environmental viewpoint, and similar disadvantages have been documented by several studies in terms of economic performance (Yadav et al., 2022). On the other hand, many high-added value products, obtained from microalgae processing, are increasingly attracting the market (Shah et al., 2016; Yadav et al., 2022). Effective strategies to enhance the sustainability of microalgal production systems are known to be related to renewable energy sources and favourable geographic locations (Prado et al., 2023), the use of wastewater (Li et al., 2022) or other waste streams (Seelam et al., 2022) as nutrient sources, and the biofixation of CO_2 from flue gas (Miranda et al., 2021).

The advancement of the technology readiness level (TRL) towards the development of industrial processes is hindered by barriers of various kinds, including the lack of sufficient data from pilot- and full-scale plants (Dębowski et al., 2022), which are essential for the conduction of reliable and accurate assessments of baseline cases and the development of market scenarios. Primary data from large-scale plants could be used to reduce the risk of misinterpretation or inaccuracies rather than secondary or even tertiary data (e.g., rough estimations from models or lab-scale extrapolated data). Additional benefits encompass the ability to reflect site-specific conditions, including details of the system, as well as local or regional features. This kind of approach also enhances transparency and replicability for stakeholders.

In a recent review article (Gurreri et al., 2023b) we conducted a detailed study of 16 LCAs on microalgal production systems, which were conducted with primary data collected at either a pilot or an industrial scale. A critical analysis of the existent literature has led to the following key points:

i. Only a few studies based on primary data from (near-)full-scale microalgal plants (i.e., with total culture volume in the order of 10 m^3 or higher) have been conducted so far. Even in these cases, downstream (i.e., post-harvesting) processing, when analysed, was primarily simulated by using data from the literature.
ii. A wide variability of results has been encountered. For example, GHG emissions spanned from 10^{-1} to 10^3 kg $CO_{2,eq}/kg_{DW}$ biomass. This can be only partially ascribed to technical aspects, such as the plant scale, the use of different algal species (each of which requires different cultivation parameter settings due to its specific physiology), bioreactors layout (open or closed), and the use of thermoregulation and/or artificial illumination.
iii. The framework becomes even much more complicated because previous studies applied different LCA methodologies in terms, for example, of functional unit, impact assessment method, and multi-product treatment. The results have also been presented in different modes. In addition, issues of information transparency were caused by missing data. All this has led to divergent results and makes it challenging to compare such studies.

More details on relevant LCA studies conducted with primary data from pilot/industrial systems are reported in the following to describe a robust background and to provide important elements for comparison purposes.

Sevigné Itoiz et al. (2012) assessed the performance of the cultivation of three marine microalgal species within bubble column photobioreactors (PBRs), each with a 99 L volume, either in indoor (artificial light) or outdoor (natural light) conditions. Energy consumption provided the main contribution to all the environmental impacts. As the outdoor cultivation provided a significant energy saving compared to the indoor one (more than 85%), it also led to a drastic reduction of the impacts and to an increase in the importance of the PBR material, which was the second contributor (~15%) in the assessed impact categories.

Pérez-López et al. (2014b) evaluated a similar pilot system, showing that the cultivation step was the most impactful for all the categories, with relative contribution ranging from 73% to 97%, mainly due to nutrients and transport, with a minor role being played by electricity consumption. Additional scenarios were simulated with alternative N sources, showing significant mitigation of the impacts.

Pérez-López et al. (2017) compared three different outdoor pilot-scale cultivation technologies provided with a heater/chiller thermoregulation system. This study showed a great influence of the bioreactor configuration and seasonality on the potential impacts, with the tubular PBRs having better environmental performances than the open raceway pond (ORP). The energy demand in the cultivation phase, especially for cooling/heating, was the most critical flow. Nutrient provision contributed significantly to a couple of impact categories, while infrastructure and waste treatment played minor roles.

Yadav et al. (2020) focused on the capture of CO_2 by testing a pilot ORP plant. They conducted a growth study either with or without an external carbon source, with the former condition being accomplished via flue gas insufflation (10% v/v CO_2), and under either a batch or semi-continuous regime. The experimental outcomes showed that the productivity was maximized for the operation with flue gas and in the semi-continuous mode. This resulted in reduced GHG emissions, as estimated via LCA. The analysis also revealed that the most significant impacts were produced by the cultivation sub-process, whose electricity consumption contributed between 75% and 85% to most impact categories. The second most relevant impact factor was either the dewatering (in the case of no flue gas) or the energy required for gas compression (in the case of flue gas insufflation).

Onorato and Rösch (2020) assessed the environmental impacts of three cultivation systems for the production of astaxanthin from *H. pluvialis*. The technologies in the reference plants were an indoor Flat Panel Airlift PBR (FPA-PBR, 93 m^3), an outdoor Unilayer Horizontal Tubular PBR (UHT-PBR, 93 m^3), and a Green Wall Panel PBR (GWP-PBR, 0.1 m^3 up-scaled to 93 m^3 in the LCA), which were installed in different climatic regions (Germany, Portugal, and France, respectively). The FPA-PBR and GWP-PBR were provided with artificial light (LEDs), while the UHT-PBR used only natural light. The LCA results showed that the main environmental hotspot was represented by the cultivation phase in the red algal stage, which contributed by 80–85% to the various impact categories. Despite the UHT system having the lowest productivity (~33% and 10% of that obtained with GWP-PBR and FPA-PBR systems, respectively) and the highest fossil fuels rate (89%), it exhibited the lowest environmental impacts, as its sunlight exposure led to a dramatic reduction in energy consumption.

The comparison conducted by Sandmann et al. (2021) between a conventional vertically stacked horizontal tubular (VSt) PBR and a mesh ultra-thin layer (MUTL) PBR at the pilot scale showed lower environmental impacts for all the categories in the former system. However, this situation was reversed when the considered functional unit was 1 mmol of specific antioxidant capacity of the biomass, due to the high biomass productivity and antioxidant capacity provided by the latter system.

Different cultivation systems, used for the production of biostimulants and aquaculture feeds, were assessed in the LCAs conducted by Herrera et al. (2021) and Pechsiri et al. (2023). A full-scale facility with 1000 m^2 of ORP was first considered (Herrera et al., 2021), but no mention was made of the microalgal species. The system exhibited very low energy consumption and environmental impacts. Among the different scenarios that were assessed, the one involving the use of wastewater for cultivation showed environmental advantages for several impact categories, including a reduction of approximately 80% in global warming and the production of negative values (i.e., credits) in several impact categories.

A comparison of four different cultivation technologies was presented by Pechsiri et al. (2023): 20 m^3 ORP, 3 m^3 Thin Layer Cascade (TLC), 4 m^3 Vertically Stacked Tubular (VSt) PBR, and 0.85 m^3 Light Exchange Bubble-column (LEB) PBR. In addition, three LEB mini reactor systems were assessed. The high electricity consumption for medium agitation (VSt-PBR) or illumination (LEB-PBR) led the environmental impacts to roughly double in the closed systems compared to the open bioreactors, and the down-scaled LEB apparatuses were even more impactful. After electricity, synthetic nutrients and CO_2 were the main impact generators for all the systems, while the reactor materials were minor contributors. The LEB system in the urban-industrial symbiosis scenario that utilized wastewater for the nutrient input, CO_2 from exhaust gases, and photovoltaic energy showed the best environmental profile, which was characterized by (i) a low climate impact and (ii) eutrophication credits.

The aim of the present study is to assess the environmental impacts of a full-scale microalgal plant (VSt-PBRs with a total volume of 40.4 m^3 cultivating *Chlorella vulgaris*) by applying the LCA methodology using only primary data of a complete foreground LCI, including both operational and construction figures. This contributes to reducing the degree of uncertainty that characterizes the available results from the pertinent literature associated with the scarcity of information sourced by large-scale facilities. By including construction materials, the inventory previously reported (Gurreri et al., 2023a) was finalized, representing a robust base to conduct an LCA study that provides a comprehensive and accurate evaluation of the environmental performance of the process across different impact categories. Moreover, a comparative analysis with previous LCA studies from the literature was performed to outline the state-of-the-art environmental performance of microalgae production technologies and the perspective for the development of sustainable bio-industries.

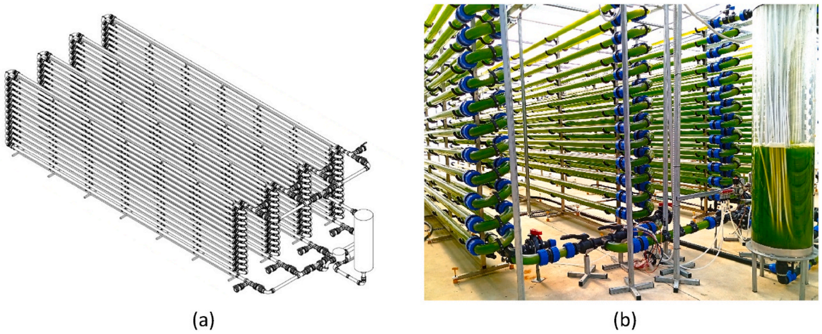
(a) (b)

Fig. 1. Vertically stacked tubular horizontal PBRs (VSt-PBRs) of the industrial-scale plant installed in Caltagirone (Italy) for the cultivation of *Chlorella vulgaris*: (a) drawing and (b) picture of a 4-loop module along with a shell and tube heat exchanger. The plant has 28 loops, for a total volume of 40.4 m^3.

Table 1
Main construction and operational features of the industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris*.

Main characteristics of the plant								
General features								
Microalgal species	Location	Occupied land [m^2]	Operating days [d/y]	Productivity [g_{DW}/L/day]	Capacity [kg_{DW}/y]	Biomass concentration post-harvesting [g/L]	Cycles [1/y]	
Chlorella vulgaris	Caltagirone, Italy	1500	300	0.08	1200	~200	5	
PBR								
Length [m]	Internal diameter [m]	Total number	Total volume [m^3]	Material	Total number of LED panels	Average growth rate [g/L/d]	Total concentration of chemicals in fresh medium [g/L]	Target biomass concentration [g/L]
10	0.082	$13 \times 2 \times 4 \times 7 = 728$	40.4	PMMA/PP	56	0.15	~8	2
Water treatment								
RO modules	N. RO modules	RO permeate flow [L/h]	RO high-pressure pump					
DuPont FILMTECTM BW30-4040	4 × 2 = 8 parallel × series	1000	8–12 bar, 2.2 kW					
Main auxiliaries								
Diaphragm-pumps	Compressor	Heat pump	Centrifuge	Piping				
8 PAW-HQ400PP	1 Atlas Copco GA55VSD+, 55 kW, 4–12.5 bar	1 CLINT CHA/K/EP 262-P, 22.3 kW	1 MACFUGE 325, 4 kW, 8300 rpm	1500 m				

2. Materials and methods

2.1. Operation and infrastructure

2.1.1. General description of the facility

The industrial-scale plant assessed in this study is installed at the Plastica Alfa company, located in Caltagirone, Sicily (37°14′55″N; 14°34′00″E), in a typical Mediterranean climatic region. The core of the plant is represented by the VSt-PBR modules (Fig. 1), which have a total volume of 40.4 m^3 and are used for the cultivation of *Chlorella vulgaris*. The plant has a volumetric productivity of 0.08 g_{DW}/L/d, a capacity of 1200 kg_{DW}/y, and an annual operativity of 300 d. The facility encompasses three different areas: i) a greenhouse (PE film) that hosts the PBRs and some auxiliaries, such as LED panels, shell and tube heat exchangers, and pneumatic pumps (double diaphragm); ii) a laterally open shed with an industrial concrete floor, which houses all the equipment used for the water treatment, a heat pump that is used for thermoregulation, a compressor and a storage vessel used to drive the pneumatic pumps and provide aeration to the PBRs, and pressurized cylinders for the supply of CO_2; and iii) a reinforced concrete building that houses a control office, an inoculum development section, and a centrifuge device. The total area occupied by the facility is ~1500 m^2.

Tap water is demineralized by means of reverse osmosis (RO) after a pre-treatment of clarification via quartzite filtration (100 μm), chlorine absorption by activated carbons, and multi-cartridge microfiltration (10 and 5 μm). The product water is used for cultivation and maintenance operations of periodic cleaning and sterilization. Four tanks (1500 L each) are used for demi water storage. Chemicals are added as nutrients for the cultivation process or as agents for cleaning and sterilization purposes. Dewatering of the algal suspension from the cultivation phase is performed via centrifugation. All the waste streams are discharged into the public sewer system.

The main features of the plant are reported in Table 1.

2.1.2. Cleaning and sterilization

The phase of cleaning and sterilization involves removing impurities, biofilms, or any accumulated substances that may affect the PBR efficiency. A chemical treatment is performed periodically to restore the optimal growth conditions in the PBRs. This operation is carried out with an average frequency of 5 times per year for each PBR module. It lasts 10 h and includes four closed-loop subphases: i) cleansing and disinfection, for 6 h, with a sodium troclosene (sodium dichloroisocyanurate) solution at 80 mg/L; ii) rinsing, for 1 h, with demineralized water; iii) cleaning and disinfection, for 2 h, with a 3% citric acid solution; and iv) final rinsing, for 1 h, with demineralized water. Each sub-phase uses almost 2000 L of demi water per PBR loop. The energy consumption is due to the treatment of tap water (especially RO) and to the compressor that drives the pneumatic pump for the solution circulation through the PBR module, ensuring, via agitation, a homogeneous washing of its internal surface. The RO retentate and the used aqueous streams are discharged into the sewer system.

2.1.3. Inoculum and cultivation

A semi-batch growth regime is applied, targeting a concentration of 2 g_{DW}/L, and the observed average growth rate is ~0.15 g_{DW}/L/d.

An initial multi-step inoculum development process is performed, ending with a system of 6 vertical airlift PBRs of 90 L each. A volume of 500 L is used to inoculate one VSt-PBR loop, while a volume of 40 L is used to restart the development of the inoculum suspension.

The cultivation is started, in a cleaned and sterilized VSt-PBR module, within three steps of (a) dilution with fresh medium and (b) batch cultivation in one, two, and four VSt-PBR loops (Fig. 1), respectively. This start-up phase lasts ~20 d and is followed by batches at a regime with 500 L/loop of fresh medium used to substitute the same amount of algal suspension when it has reached the target concentration. On average, each batch operation lasts ~6 d, and there are 6.4 batches per cycle and 32 batches per year in each VSt-PBR module. The volume of the bioreactors (40.4 m^3 in total) is filled by 70% with the algal suspension, which is also present in the shell side of the heat exchangers. The transparent material used for the bioreactors is poly(methyl methacrylate) (PMMA).

The culture medium is prepared by adding commercial chemical nutrients, at a total concentration of ~8 g/L, to demineralized tap water. The water treatment section (RO and pre-treatments) is aimed at removing any impurities, salts, and/or other unwanted substances that could interfere with the setting of the nutritional parameters or pH and with the productivity of the bioreactor. The chemicals are introduced in a tank where the fresh medium is circulated during UV sterilization, which is applied to further enhance its safety in terms of contamination. Commercial CO_2 for microalgae photosynthesis and pH control is insufflated into the bioreactors by pressurized cylinders. Mixing and aeration are provided inside the bioreactors by air insufflation, while pneumatic pumps are used for the circulation of the algal suspension in the VSt-PBRs, both operations being accomplished by an air compressor (Atlas Copco GA55VSD+) and a steel vessel used for storage. Natural illumination is used during the daytime, while artificial LED lighting is used during dark hours, thus a continuous photosynthetic activity is basically maintained. A thermoregulation system is used to ensure an optimal growth temperature over the 21 °C–27 °C range. The main components of this system are an industrial heat pump (Clint CHA/K/EP 262-P) that allows the heating and cooling to be modulated on the basis of seasonal temperature variations, and tube bundle heat exchangers, which are installed to control the temperature in the VSt-PBRs.

2.1.4. Harvesting

The algal suspension produced in the VSt-PBRs at 2 g_{DW}/L with an average flow rate of ~1650 L/d is sent to a dewatering phase for microalgae harvesting in the form of a highly concentrated suspension (up to 200 g_{DW}/L). This is accomplished by centrifugation (Macfuge 325), which is operated for ~1 h/d. The exhaust culture medium is discharged into the sewer system, without any recirculation.

2.2. *Life cycle assessment methodology*

This LCA study was implemented with a methodology that follows the principles and guidelines set out in the international standard norms ISO 14040 (2006) and ISO 14044 (2006). The raw primary data obtained from the industrial plant were elaborated in Excel spreadsheets to create the foreground inventory. SimaPro software, v. 9.5.0.0, was used to model the product system by creating its background inventory and assessing its potential environmental impacts.

2.2.1. *Goal and scope definition*

The goal of the present study was to assess the potential environmental impacts of the production of *C. vulgaris* biomass in an industrial-scale plant that uses the VSt-PBRs technology and is located in Caltagirone, Italy. A thorough LCA was conducted with primary data to comprehensively describe the environmental performance of the analysed production system and to identify its environmental bottlenecks in terms of impactful process stages and input/output flows.

The "cradle-to-gate" approach was adopted with the functional unit (FU) of "1 kg of dry-weight biomass". The assessment was based on the collection of field data on the operation and infrastructure of the plant, including (material and energy) resource consumption, waste production, and emissions. The transport of materials was included in the analysis by using background data for Italian, European, or global markets (in decreasing order of preference). The lifetime of the plant was assumed to be 30 years, and thus the temporal boundary of the analysis was defined. The location of the facility and the use of the Italian grid mix for electricity were the main items identifying the geographical boundary.

To enable an insightful assessment of the impact contributions, the product system was modelled in two alternative configurations (Fig. 2). In arrangement A, four sub-processes were used to represent the infrastructure (including all the equipment and machinery) and the operating steps of the plant (Fig. 2a). In layout B, the sub-processes were devised to represent the different "flow types" (Fig. 2b).

After biomass harvesting (dewatering), different downstream processes may be applied to obtain various final products, but such processes have not been considered in this study. However, a very simple downstream process could be that of ultrasonication for cell disruption, which produces biostimulants.

2.2.2. *Life cycle inventory: data elaboration and implementation*

The quality of the data used for the life cycle inventory (LCI) phase is extremely important to obtain results that closely reflect reality and minimize uncertainty. In the present study, the LCI compilation was carried out almost exclusively using primary data from an industrial-scale facility that cultivates *C. vulgaris*. The data retrieval covered one year of operation, and thus included seasonal variations.

The foreground inventory was built by collecting and elaborating raw field data in spreadsheets, which incorporate the operational and infrastructural features of the real plant described in section 2.1 and reflect the modelled product system depicted in Fig. 2. An annual operating time of 300 days was considered, along with 5 complete cleaning and cultivation cycles per year. First, average operational data were elaborated (through mass balances and other basic calculations) for one cultivation cycle and one VSt-PBR module, while the components of the infrastructure were inventoried for the whole facility. All the input and output flows were then calculated for the chosen FU (1 kg_{DW} biomass), assuming a lifespan for all the infrastructural components (30 years for buildings and piping and 20 years for all other main auxiliaries).

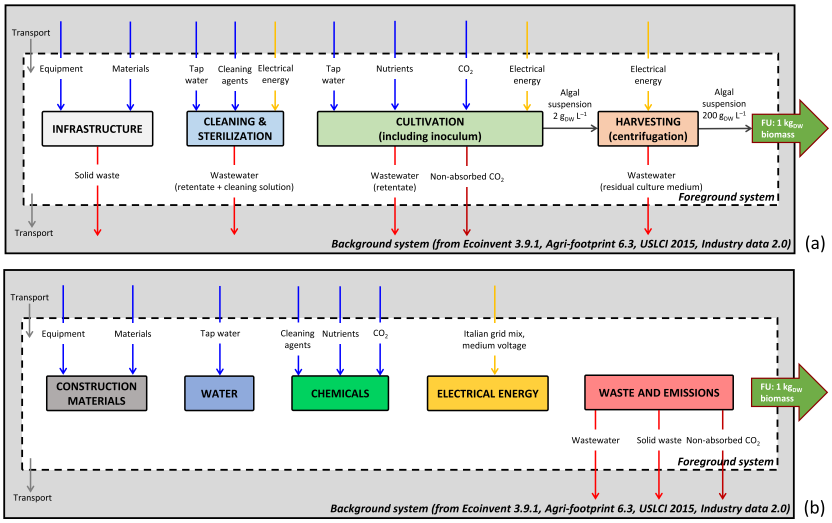

Fig. 2. Schematic diagrams of the product system with the boundary, foreground, background, and input/output flows through the sub-processes used to model the industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris*: (a) configuration with the infrastructure and operational stages; (b) configuration with the flow type sub-processes.

The product system was built in the SimaPro environment by implementing all the input/output flows from/to the techno-sphere and the waste flows to the treatment. The product system in configuration A was divided into the four sub-processes of (i.a) infrastructure, (ii.a) cleaning and sterilization, (iii.a) cultivation (including inoculum), and (iv.a) harvesting (centrifugation) (Fig. 2a). The same system was divided into five sub-processes in configuration B, four of which regard the input flows of (i.b) construction materials, (ii.b) tap water, (iii.b) chemicals (nutrients + cleaning and sterilization agents + CO_2), and (iv.b) electrical energy (Italian grid mix, medium voltage), and one sub-process regarding (v.b) waste and emissions, where all the outputs (wastewater + solid waste from construction materials + non-absorbed CO_2 emitted into the air) are grouped (Fig. 2b). The RO retentate, the waste solution from cleaning and sterilization of the bioreactors, and the residual culture medium from centrifugation were modelled as wastewater discharged into the sewer system and sent to conventional wastewater treatment. The waste from the construction materials was simulated as solid waste (concrete, plastic mixture, scrap steel, and inert waste) that is disposed of in either a landfill for inert materials or in a sanitary landfill, depending on the waste typology.

The professional Ecoinvent (v. 3.9.1), Agri-footprint (v. 6.3), USLCI 2015, and Industry data 2.0 databases were used to implement the input/output flows and to obtain the inventory data of the background system. Whenever possible, “market” processes were chosen to include average background data on the transport of materials. These processes were primarily related to the European level and, when available, to the Italian one (e.g., Italian grid mix, medium voltage for electricity). Some chemicals were not included in the

Table 2

Foreground LCI reporting the main flows of the industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris*. All the quantities refer to the FU of 1 kg_{DW} biomass and are grouped in sub-tables reflecting the structure of the product system in configuration A (Fig. 2a). Data previously published (Gurreri et al., 2023a) were updated (energy consumption for thermoregulation) and integrated with the infrastructure sub-process.

Sub-process	Material or energy input from the technosphere	Unit of input flow	Value of input flow	Material output to the technosphere or emission to the environment	Unit of output flow	Value of output flow
1. Infrastructure						
	PMMA for PBRs	g	405	Concrete (to inert landfill)	g	34,127
	Fittings, valves, faucets, and pneumatic pumps	g	172	Plastic mixture (to sanitary landfill)	g	904
	Piping	g	201	Scrap steel (to inert landfill)	g	501
	Stainless steel for the PBR chassis and the greenhouse frame	g	250	Inert waste (to inert landfill)	g	4552
	Steel for the open shed frame	g	222			
	Greenhouse	g	83			
	Concrete	m^3	0.0142			
	Bricks	g	4356			
	Compressor	g	37			
	Heat pump	g	38			
	Centrifuge	g	13			
	LED	g	12			
	RO modules	m^2	0.0024			
2. Cleaning and sterilization						
	Tap water	L	563	Wastewater	L	563
	Sodium troclosene	g	10			
	Citric acid	g	3797			
	Pumping and agitation	kWh	2			
3. Cultivation (including inoculum)						
	Tap water	L	556	Wastewater	L	56
	$Na_2MoO_4 \cdot 2H_2O$	g	33	CO_2 (emission)	g	500
	$CaCl_2 \cdot 2H_2O$	g	167	O_2 (emission)	g	2000
	$Na_2EDTA \cdot 2H_2O$	g	83			
	$FeSO_4 \cdot 7H_2O$	g	167			
	K_2HPO_4	g	333			
	K_2SO_4	g	333			
	$MgSO_4 \cdot 7H_2O$	g	333			
	$MnCl_2 \cdot 4H_2O$	g	33			
	NaCl	g	333			
	$NaHCO_3$	g	1667			
	$CuSO_4 \cdot 5H_2O$	g	333			
	H_3BO_3	g	167			
	$ZnSO_4 \cdot 7H_2O$	g	167			
	CO_2	g	2500			
	Thermoregulation	kWh	108			
	Pumping and agitation	kWh	115			
	Lighting	kWh	40			
4. Harvesting (centrifugation)						
	Centrifugation	kWh	1.4	Wastewater	L	495

database. In these cases, proxy processes with similar compounds were chosen.

2.2.3. *Life cycle impact assessment methods*

The life cycle impact assessment (LCIA) was performed using the CML-IA baseline (v. 3.09/EU25) method. The CML method, developed by researchers from the University of Leiden, the Netherlands, contains characterization factors in a problem-oriented approach (midpoint modelling) for all the baseline methods mentioned in Nygren et al. (2002). The eleven impact categories of the CML baseline method are: i) abiotic depletion (ADP), ii) abiotic depletion (fossil fuels) (ADPF), iii) global warming for a time horizon of 100 years (GWP100a), iv) ozone layer depletion (ODP), v) human toxicity (HTP), vi) freshwater aquatic ecotoxicity (FAETP), vii) marine aquatic ecotoxicity (MAETP), viii) terrestrial ecotoxicity (TETP), ix) photochemical oxidation (POP), x) acidification (AP), and xi) eutrophication (EP). In the present study, the LCIA was limited to the characterization phase, and normalization was omitted.

2.2.4. *Interpretation*

The interpretation phase of the LCA is the step in which the results are examined, evaluated, and critically interpreted in order to draw conclusions that can then be used to guide decision-making to obtain more sustainable options. The presentation of the results of the LCI and LCIA of this study was structured to facilitate their interpretation in the identification of the most significant process stages and the input/output flows in terms of resource consumption, waste production, and potential environmental impacts. This approach revealed the environmental hotspots that require more attention and for which improvements should be proposed to develop more sustainable systems. Some comparisons with the results of other LCA case studies from the literature were crucial to provide a broader picture, draw conclusions, and outline future perspectives.

3. Results and discussion

3.1. *Life cycle inventory analysis*

Table 2 shows the foreground inventory of the analysed industrial plant, which was based on operational and infrastructural features and created exclusively with primary data. All the inputs and outputs were normalized to the FU of 1 kg of dry-weight biomass.

The main inputs required by the infrastructure sub-process are concrete for buildings (0.0142 m^3/kg_{DW}), steel for frame structures (472 g/kg_{DW} in total), and PMMA for PBRs (405 g/kg_{DW}). The inventory analysis also highlights a large consumption of chemicals, with a total amount of ~4 kg/kg_{DW} in cleaning and sterilization (almost exclusively citric acid) and a similar amount in cultivation (sodium bicarbonate, nitrogen- and phosphorous-based nutrients, and other chemical fertilizers). The process is also quite energy-intensive, as electricity consumption amounts to 267 kWh/kg_{DW}, mainly due to operations performed in the cultivation step, i.e.,

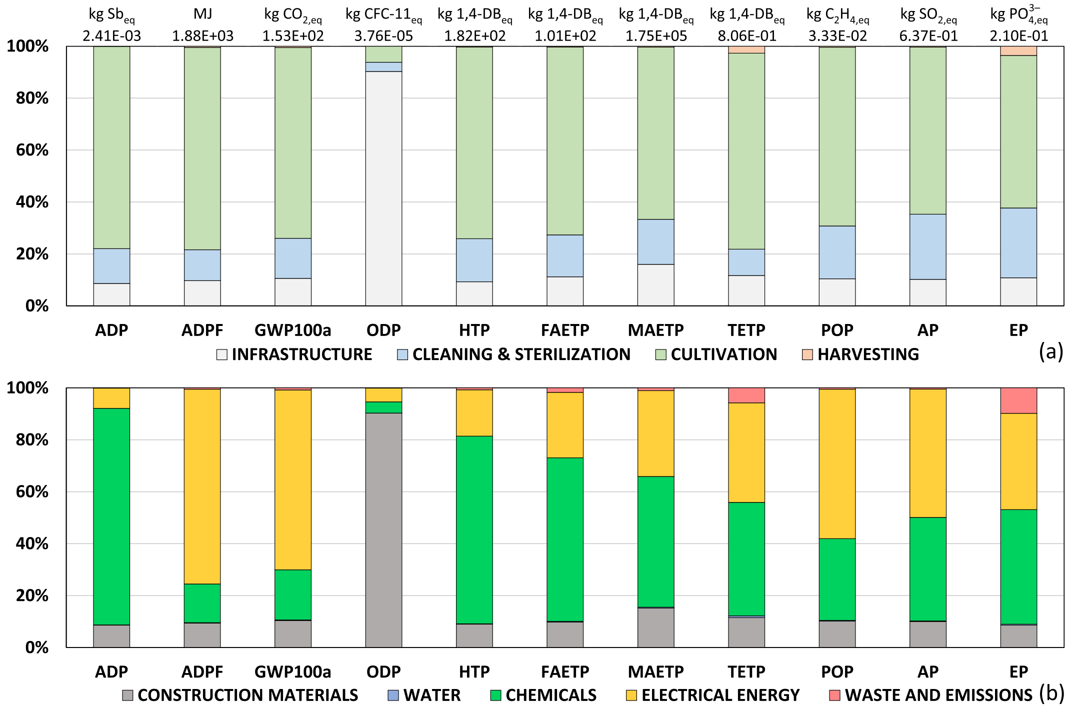

Fig. 3. LCIA results obtained with the CML-IA baseline method for the industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris* – relative contributions to the environmental burdens of (a) the infrastructure and operational stages (product system in configuration A, Fig. 2a), and (b) the flow type sub-processes (product system in configuration B, Fig. 2b). Abiotic depletion (ADP), abiotic depletion (fossil fuels) (ADPF), global warming for a time horizon of 100 years (GWP100a), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FAETP), marine aquatic ecotoxicity (MAETP), terrestrial ecotoxicity (TETP), photochemical oxidation (POP), acidification (AP), eutrophication (EP).

(i) pumping and agitation and (ii) thermoregulation, followed by LED lighting in dark hours. Regarding the outputs of the product system, solid waste from infrastructure and wastewater from the cleaning and harvesting process stages are the main flows.

3.2. Life cycle impact assessment: characterization results

The LCIA outcomes are summarized in Fig. 3. It reports, along with the characterization results, the breakdown of the contributions that depicts the environmental profile of the analysed microalgal process system, identifying the environmental hotspots in terms of process stages and flow types.

Fig. 3a shows that the cultivation is the most impactful process stage, providing relative contributions from ~59% to 78% across the various impact categories, apart from Ozone Layer Depletion (ODP), in which the cultivation phase contributes only by 6%. The cleaning and sterilization step provides milder relative contributions falling in the range of ~10–27%, with an exceptionally low value of ~4% for ODP. An opposite behaviour is exhibited by the infrastructure subprocess, which dominates in the ODP impact with an incidence of 90% while providing modest contributions in the range of ~9–16% in all other impact categories. A contribution analysis of the flows grouped in the infrastructure subprocess revealed that its ODP is almost totally caused by the RO modules. As expected according to the inventory analysis, the role played by the harvesting phase is almost negligible, as this subprocess is characterized only by small amounts of energy consumption and wastewater production.

Fig. 3b reveals that the consumption of chemicals is the most important flow across the assessed impact categories, providing the largest contribution (from 44% to 83%) in 6 of them. The consumption of electrical energy from the grid is the second source of environmental impact generation, as it is the leading sub-process in 4 impact categories (incidence from 49% to 75%). These results reflect the high amount of cleaning agents and nutrients (~4 kg/kg_{DW} for both of them as total input, see section 3.1) and the high energy consumption (267 kWh/kg_{DW} mostly related to the cultivation stage) of the product system. Again, a special behaviour is observed in the ODP category, which shows a net predominance of the construction materials subprocess as an impact generator (~90% contribution) due to the RO modules, as mentioned above. The subprocess of waste treatment and direct emissions produces relatively modest potential impacts (contribution below 10%), while the consumption of tap water has negligible effects.

Aiming at further quantifying the importance of the main flow types in terms of potential environmental impacts, the LCIA results were elaborated to conduct a single-factor sensitivity analysis. It reflects a 25% variation in the amount of three different input sub-processes (one at a time): a) chemicals, b) electrical energy, and c) construction materials. Resource consumption and biomass productivity in microalgae cultivation are quite sensitive to environmental conditions (Pérez-López et al., 2017), and variations of ±25% can be considered realistic in normal operations at the facility (Pechsiri et al., 2023). The results are reported in Table 3 as percentage variations relative to the baseline value.

A 25% variation in the chemicals consumption for PBR cleaning and microalgae growth, which is the most influential sub-process, would result in an impact variation higher than 15% in the categories of ADP, HTP, and FAETP. Overall, similar yet milder effects are produced by the consumption of electricity from the grid, which is the second impact driver. In particular, a 25% variation in energy demand would result in an impact variation higher than 15% in the categories of ADPF and GWP100a. Finally, the effect of perturbing inputs related to construction materials shows that important effects would occur only for the ODP impact category, as expected according to the LCIA results discussed above, with an incidence of 22.6%.

3.3. Comparative analysis with data from the literature

To perform a comparison with other case studies from the literature, Table 4 reports the main features of recent LCA studies conducted with primary data at the pilot or industrial scale. From the analysis of Table 4 and it can be highlighted that cultivation plants used for data provision have been based on different technologies, with a predominance of closed PBR systems. Moreover, they have been developed at different scales, ranging from a minimum of 80 L to a maximum of 93 m^3 of total culture volume. It should be

Table 3

Sensitivity analysis obtained by a variation of 25% for three subprocesses devised per input flow type in the industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris*: electrical energy, chemicals, and construction materials (see Fig. 2b). The perturbation effect is expressed as a percentage variation relative to the baseline value for each impact category of the CML method.

Impact category	Baseline value	Effect of chemicals [%]	Effect of electrical energy [%]	Effect of construction materials [%]
Abiotic depletion (ADP)	2.39E-03 kg Sb_{eq}	20.8	2.0	2.2
Abiotic depletion (fossil fuel) (ADPF)	1.87E+03 MJ	3.7	18.8	2.3
Global warming for a time horizon of 100 years (GWP 100a)	1.53E+02 kg $CO_{2,eq}$	4.8	17.3	2.6
Ozone layer depletion (ODP)	3.76E-05 kg CFC-11_{eq}	1.1	1.3	22.6
Human toxicity (HTP)	1.77E+02 kg 1,4-DB_{eq}	18.1	4.5	2.2
Freshwater aquatic ecotoxicity (FAETP)	1.00E+02 kg 1,4-DB_{eq}	15.8	6.3	2.5
Marine aquatic ecotoxicity (MAETP)	1.73E+05 kg 1,4-DB_{eq}	12.6	8.3	3.8
Terrestrial ecotoxicity (TETP)	7.89E-01 kg 1,4-DB_{eq}	10.9	9.6	2.9
Photochemical oxidation (POP)	3.29E-02 kg $C_2H_{4,eq}$	7.9	14.4	2.5
Acidification (AP)	6.33E-01 kg $SO_{2,eq}$	10.0	12.4	2.5
Eutrophication (EP)	2.08E-01 kg $PO_{4,eq}^{3-}$	11.0	9.3	2.2

Table 4
Main features of (i) pilot- and industrial-scale microalgal plants providing primary data and (ii) LCA methodologies in studies conducted with the FU of 1 kg_{DW} biomass. "N.A." means "not available".

Microalgal species	Bioreactor configuration	Bioreactor or culture volume [m^3]	Location (Country)	LCA software	Database	LCIA method	Reference
1) *Alexandrium minutum* 2) *Karlodinium veneficum* 3) *Heterosigma akashiwo*	Bubble column (BC) PBR, either illuminated by fluorescent lights and thermoregulated at room temperature (indoor) or not (outdoor)	0.099 (per each algal species)	Barcelona (Spain)	SimaPro 7.1.8	Ecoinvent (version N.A.)	CML baseline 2001 (version N.A.), CED 1.4	Sevigné Itoiz et al. (2012)
Tetraselmis suecica	Vertical BC-PBR, illuminated by cool white and fluorescent light, thermoregulated, indoor	0.08	N.A. (probably Galicia, Spain)	SimaPro 7.3	Ecoinvent 2.0	CML baseline 2001 2.04	Pérez-López et al. (2014b)
Nannochloropsis sp.	1) Horizontal (Hor) PBR 2) Vertically stacked (VSt) PBR 3) Open raceway pond (ORP) Thermoregulated systems (heater/chiller), outdoor	1) 0.56 2) 1.06 3) 4.73	Wageningen (Netherlands)	SimaPro 8	Ecoinvent (version N.A.)	CML 2001 (version N.A.), CED (version N.A.)	Pérez-López et al. (2017)
Chlorella vulgaris	ORP, outdoor	0.12	Kharagpur (India)	SimaPro 8.0.3.14	Ecoinvent 2.2	IPCC-GWP 2013, CML 2 baseline 2000, CED (version N.A.), ReCiPe midpoint (H) and endpoint (H) (version N.A.)	Yadav et al. (2020)
Haematococcus pluvialis	1) Green wall panel (GWP) PBR, illuminated by LEDs, outdoor 2) Flat panel airlift (FPA) PBR, illuminated by LEDs and ventilated for thermoregulation, indoor 3) Unilayer horizontal tubular (UHT) PBR, cooled by freshwater spraying, outdoor	1) 0.1 2) 93 3) 93	1) Montpellier (France) 2) Stuttgart (Germany) 3) Lisbon (Portugal)	OpenLCA 1.6	Ecoinvent 3.3	ReCiPe midpoint (H) 2014	Onorato and Rösch (2020)
Acutodesmus obliquus	1) VSt-PBR, cooled by water sprinkling, in a greenhouse 2) Mesh ultra-thin layer (MUTL) PBR, water-cooled, in a greenhouse	1) 0.25 2) 0.1	Nuthetal (Germany)	SimaPro 8.2.0.0	Ecoinvent 3.1, Agri-footprint (version N.A.)	IMPACT 2002+ midpoint and endpoint 2.21	Sandmann et al. (2021)
N.A.	ORP, outdoor	200[a]	Almería (Spain)	SimaPro 9.0.0.41	Ecoinvent 3.5	ReCiPe midpoint (H) 2016 1.13	Herrera et al. (2021)
Scenedesmus almeriensis	1) ORP, outdoor 2) Thin layer cascade (TLC), in greenhouse 3) VSt-PBR, in a greenhouse 4) Light exchange bubble-column (LEB) PBR, illuminated by LED tubular grid, indoor 5) LEB mini reactors: a. MR-1 b. MR-2 c. MR-3	1) 20 2) 3 3) 4 4) 0.85 5) a. 0.36 b. 0.36 c. 0.35	Almería (Spain)	SimaPro 9	Ecoinvent 3.3, USLCI 2015, ELCD (2016)	CML 2001 3.04, CED 1.09	Pechsiri et al. (2023)
Chlorella vulgaris	VSt-PBR, illuminated by LEDs and thermoregulated (heat pump), in a greenhouse	40.4	Caltagirone (Italy)	SimaPro 9.5.0.0	Ecoinvent 3.9.1, Agri-footprint 6.3, USLCI 2015, Industry data 2.0	CML-IA baseline 3.09	This work

[a] Estimated assuming a height of culture medium in the ORP of 0.2 m as in Pechsiri et al. (2023).

stressed that, among the various microalgae cultivation systems assessed in previous studies, only a few plants are comparable in scale with the industrial VSt-PBR facility evaluated in the present work, i.e., the FPA-PBR and UHT-PBR systems assessed by Onorato and Rösch (2020), and the ORP systems assessed by Herrera et al. (2021) and Pechsiri et al. (2023). Some heterogeneity can be also observed in the LCA methodologies, although there is a predominance in the use of the SimaPro software, the Ecoinvent database, and the CML method. Nevertheless, updates in the used databases introduce variability in the results, and the deployment of different LCIA methods makes full comparisons of characterization results impossible and affects the values of analogous categories.

By taking into consideration the due caution in making comparisons, Fig. 4 depicts normalized figures of merit representative of the technical and environmental performance of microalgal cultivation plants. From the LCA studies that assessed multiple scenarios, one or more representative base case studies were selected for comparison purposes, while "virtual" scenarios were neglected. Fig. 4 highlights that the performance metrics of pilot- and industrial-scale microalgal plants exhibit a huge variability. Across the various case studies, volumetric productivity, chemicals consumption, electrical energy consumption, and GHG emissions span in the wide ranges of 0.013–1.43 g_{DW}/L/D, 8.6–8103 g/kg_{DW}, 1–5738 kWh/kg_{DW}, and 1.5–4256 kg $CO_{2,eq}$/kg_{DW}, respectively.

Note that some data are not available. However, from the results reported in Fig. 4, it can be asserted that the VSt-PBR industrial plant assessed in this study is characterized by a middle-low value of productivity (0.08 g_{DW}/L/d). To increase the techno-environmental performance of this plant, its biomass productivity should be enhanced, for example, by optimizing the operating conditions. Indeed, productivity could be a crucial indicator for the environmental performance of microalgal cultivation, as high values can be associated with resource-efficient and low-impact profiles. Nevertheless, across the various data from the different case studies, there is no clear correlation between productivity versus (material and energy) inputs or GHG emissions. This can be widely attributed to the different technologies and scales of the assessed microalgal plants. On the other hand, the high or even extremely high values of productivity (up to 1.43 g_{DW}/L/d, i.e., 18 times the productivity of the VSt-PBR industrial plant assessed in the present study) that characterize the microalgal systems from Pechsiri et al. (2023) are associated with a good environmental performance.

Regarding the chemicals consumption, which was identified as the main impact driver by the LCIA (section 3.2), Fig. 4 highlights that the amount characterizing the present VSt-PBR plant (almost 8 kg/kg_{DW}, section 3.1) is the highest one together with that from the VSt-PBR case study from Pérez-López et al. (2017). This suggests the opportunity for a re-evaluation of the operational quantities of fertilizers as well as cleaning and sterilization agents, which should be minimised without compromising the productivity, the absence of contamination of the culture medium, and the algal composition (carbohydrate, lipid, and protein content) required for the desired final product. The possibility of reducing significantly the chemical consumption is widely corroborated by the studies illustrated in Fig. 4, reporting values of chemical demand up to ~100 times lower (or even below) for microalgal plants with different bioreactor configurations, algal species, and water types (freshwater or seawater). In this regard, combining wastewater treatment or the valorisation of other waste streams (e.g., manure or digestate) with microalgae cultivation could have great potential for impact mitigation, as demonstrated by several case studies (additional scenarios not included in Fig. 4 for the sake of brevity). However, despite integrated strategies could be effective in sustainability enhancement, their applicability is limited by constraints on the final use (obviously, nutraceutical, food and feed products must be excluded). Aiming at generating lower potential impacts, another option is to evaluate the use of alternative chemicals as microalgal nutrients (Pérez-López et al., 2014b) or cleaning and sterilization agents.

By totalling 267 kWh/kg_{DW} (section 3.1), the electrical energy demand, which is the other environmental hotspot for impact generation (section 3.2), exhibits a middle-high value compared to the data from the literature (Fig. 4). As shown in Table 2, electricity

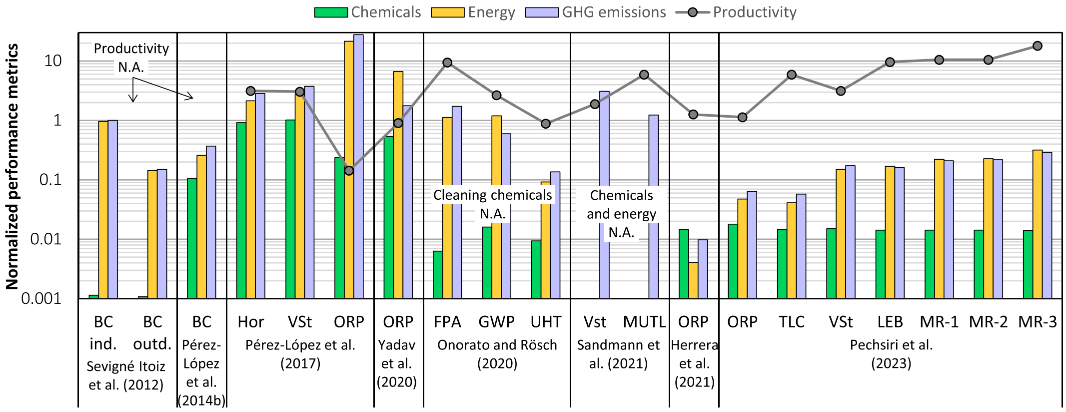

Fig. 4. Technical and environmental performance of pilot- and industrial-scale microalgal plants assessed in LCA studies based on primary data and conducted with the FU of 1 kg_{DW} biomass. Literature data are normalized to the values from the present case study (industrial-scale VSt-PBRs plant for the cultivation of *Chlorella vulgaris*): productivity of 0.08 g_{DW}/L/d, chemicals consumption of 7958 g/kg_{DW} (CO_2 excluded), energy consumption of 267 kWh/kg_{DW}, and GHG emissions of 153 kg $CO_{2,eq}$/kg_{DW}. "N.A." means "not available". From Sevigné Itoiz et al. (2012), data for the cultivation of *Alexandrium minutum* are considered, as there were no substantial differences in the results obtained from any of the marine microalgae studied. From Pérez-López et al. (2014b), virtual scenarios with alternative N sources were omitted. From Pérez-López et al. (2017), data on operation during fall are selected as representative of average values over all seasons. From Yadav et al. (2020), the best case is reported, i.e., with flue gas insufflation in a semi-continuous cultivation regime. From Onorato and Rösch (2020), the FPA-PBR plant with the German grid mix is considered, while the same system with the French grid mix is omitted. From Herrera et al. (2021), the scenario with freshwater and fertilizers without recirculation is selected (other scenarios exhibited even lower values of inputs and impacts). From Pechsiri et al. (2023), virtual scenarios of urban-industrial symbiosis (flue gas and wastewater) and locally installed solar energy were omitted.

is mainly required for pumping and aeration (43%), thermoregulation (40%), and LED lighting (15%). This qualitatively confirms previous results about (i) the relatively high energy consumption for agitation that characterizes VSt-PBR systems (Pechsiri et al., 2023) and (ii) the prominent influence of temperature control on the energy demand and its environmental impacts (Pérez-López et al., 2017). On the other hand, micro-climatic conditions favourable to microalgal growth without energy-intensive thermoregulation and lighting systems would be preferable (Pechsiri et al., 2023). Other improvements can be achieved by the deployment of optimized systems for pumping and aeration.

Some correlation can be observed in Fig. 4 between energy consumption and GHG emissions, as can be expected when electricity is harvested from Country grid mixes with a low share of renewable energy. Similarly to the behaviour of the energy demand, the global warming potential generated by the assessed VSt-PBR industrial plant exhibits a middle-high value (153 kg $CO_{2,eq}/kg_{DW}$) in the comparative analysis. By focusing on VSt-PBR systems only, this result is between the values from Pechsiri et al. (2023) and Pérez-López et al. (2017). In the prospect of future enhancements of the environmental profile of the assessed plant, any improvement in energy efficiency will result in a mitigation of impacts (not only GPW100a but also ADPF and other categories according to Fig. 3b). A powerful strategy for the reduction of GHG emissions suggested by Pechsiri et al. (2023) can be the deployment of locally installed photovoltaic panels. However, the well-known toxicity of their materials (Li et al., 2024) imposes a comprehensive evaluation of different impact categories.

To complete the comparison of LCA studies, a further brief analysis can be made in terms of main impact generators. Overall, the literature review included in the Introduction highlights that energy consumption tends to be the main impact driver. It is followed by nutrient provision, while other contributors such as construction materials and waste treatment play a minor role. In contrast, the LCIA characterization conducted in this study (section 3.2) revealed that, for the present industrial VSt-PBR plant, the consumption of chemicals (for cultivation and maintenance) is the most important flow across the assessed impact categories. However, energy consumption has a similar relevance.

Finally, in light of the heterogeneity of the methodological approaches and the unavailability of some data among previous LCA studies for microalgal systems, we want to claim, in agreement with Pechsiri et al. (2023), the need for harmonisation and improved transparency in future studies.

4. Conclusions

This study presents a comprehensive LCA of an industrial-scale production site of microalgal biomass by using exclusively primary data, including the infrastructure, for the compilation of the foreground inventory. Data were collected at the facility of an outdoor VSt-PBR system (~40 m^3 culture volume) located in Sicily for the production of *Chlorella vulgaris*.

The inventory analysis highlighted a large consumption of chemicals (almost 8 kg/kg_{DW}), of which ~50% for maintenance (cleaning and sterilization) and 50% for cultivation, and a high electrical energy demand (267 kWh/kg_{DW}) mainly due to pumping and aeration (43%), thermoregulation (40%), and LED lighting in dark hours (15%). The main infrastructure requirements were concrete for buildings (~34 kg/kg_{DW}), steel (~0.5 kg/kg_{DW}), and PMMA for PBRs (0.405 kg/kg_{DW}). The impact assessment (CML method) revealed that cultivation is by far the most impactful process stage, followed by maintenance and infrastructure, while the contribution of harvesting is almost negligible. The breakdown of the contributions of the different flow types highlighted that the consumption of chemicals and electricity from the grid represented the main environmental hotspots. In contrast, construction materials and waste treatment played only a minor role in the life cycle of the microalgal plant (apart from the high share of ozone layer depletion due to construction materials). The potential GHG emissions were ~150 kg $CO_{2,eq}/kg_{DW}$, highlighting poor sustainability in the framework of carbon-neutral production systems. However, this result was aligned with several studies from the recent literature, in which the global warming potential estimations are scattered over several orders of magnitude, spanning from 1 to more than 1000 kg $CO_{2,eq}/kg_{DW}$.

Further research is needed to improve the sustainability of microalgal production systems. Beyond the process intensification (enhancement of productivity), the use of nutrient-rich wastewater and the adoption of renewable energy could lead to significant mitigation of environmental impacts, with possible improvements also in the process economics.

CRediT authorship contribution statement

Luigi Gurreri: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mirko Calanni Rindina:** Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Antonella Luciano:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation. **Luciano Falqui:** Resources, Investigation, Data curation, Conceptualization. **Debora Fino:** Writing – review & editing, Writing – original draft, Validation, Supervision. **Giuseppe Mancini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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