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Life Cycle Inventory Based on Primary Data of an Industrial Plant for the Cultivation of *Chlorella Vulgaris*

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Microalgae and cyanobacteria represent a valuable resource with great potential in the production of a large variety of high-added value products in the nutraceutical, cosmetic, pharmaceutical, and food sectors. However, the commercialization of microalgal systems and biorefineries is limited by environmental and economic sustainability concerns. Many life cycle assessments (LCAs) have been conducted so far, but they have the crucial limitation of using data extrapolated from lab-scale experiments or the literature, thus providing qualitative and uncertain projections. In this work, an industrial-scale plant's life cycle inventory (LCI) is compiled with primary data and analyzed. The plant is installed in Sicily (Italy) and has a 1200 kgDW year^{-1} capacity in the production of *Chlorella vulgaris* microalgae. The cultivation is performed in vertically stacked horizontal photobioreactors (PBRs) with a total volume of 40.4 m^3. Dewatering is performed by centrifugation, producing a final algal suspension at ~200 g_{DW} L^{-1}. Energy, nutrients, water, chemicals, and infrastructure materials are inventoried to characterize the operation and construction. One kg of dry algae biomass is the functional unit chosen to report all elementary input/output flows. The results are compared with relevant LCIs from the literature, providing a picture of the current status of microalgae production in large-scale plants.

1. Introduction

Microalgae have a huge potential for a multitude of production routes, including biofuels/bioenergy (Marangon et al., 2023), biomaterials (Nanda and Bharadvaja, 2022), and valuable biochemicals (Braun and Colla, 2022). However, the economic (Yadav et al., 2022) and environmental (Ubando et al., 2022) sustainability of microalgal production and biorefinery is uncertain and presents several challenges, hindering the competitiveness against established technologies. Many research efforts have been devoted to the life cycle assessment (LCA) of microalgal systems, especially in the last decade. Nevertheless, LCA studies have been conducted mostly with data extrapolated from lab-scale setups or even with literature data. In contrast, few studies have been based on primary data from large-scale plants (Gurreri et al., 2023). Moreover, LCAs of microalgal systems used different methodologies (e.g., functional units), and some of them show a lack of transparency and clarity, even in the life cycle inventory (LCI). It consists of collecting and analyzing all input/output flows (resource consumption, emissions, and wastes), representing a crucial step in the LCA.

Industrial-scale plants evaluated by LCA studies are listed in Table 1 along with some examples of pilot-scale installations. Tubular photobioreactors (PBRs) or open raceway ponds (ORPs) are the most common cultivation systems of microalgae. Pérez-López et al. (2017) compared three pilot bioreactors, i.e., a horizontal PBR, a vertically stacked PBR, and an ORP, for the cultivation of *Nannochloropsis* sp. Data were collected during summer, fall, and winter in Wageningen, the Netherlands, and all systems were thermoregulated. The LCI showed large effects of seasonality and bioreactor configuration. The main inputs were ~0.4-9.0 kg kg^{-1} for

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construction materials (mainly aluminum, PMMA, and PP for the Hor, VSt, and ORP reactor, respectively), 128-15984 L kg_{DW}^{-1} for tap water (cleaning), 426-15628 L kg_{DW}^{-1} for seawater (cultivation), ~0.9-6.6 kg kg_{DW}^{-1} for $NaNO_3$ (major nutrient), 559-~12.000 g kg_{DW}^{-1} for cleaning chemicals, and ~280-5600 kWh kg^{-1} for energy consumption with major contributions due to heating/cooling and aeration.

Onorato and Rösch (2020) compared three commercial-scale PBR plants (93 m^3): an indoor Flat Panel Airlift (FPA) PBR, illuminated by LEDs and ventilated for temperature control; an outdoor Green Wall Panel (GWP) PBR (up-scaled data), illuminated by LEDs; an outdoor Unilayer Horizontal Tubular (UHT) PBR, cooled by freshwater spraying. The cultivation of *Haematococcus pluvialis* and the extraction of astaxanthin were studied. The FPA, GWP, and UHT PBR plants were located in Stuttgart (Germany), Montpellier (France), and Lisbon (Portugal), respectively. Their main inputs for microalgae cultivation were 346, 556, and 1574 L kg_{DW}^{-1} for water, 50, 127, and 75 g kg_{DW}^{-1} for nutrients, and 300, 318, and 25 kWh kg_{DW}^{-1} for electricity, respectively.

Herrera et al. (2021) assessed a 1000 m^2 ORP installed in Almería (Spain) and producing biostimulants. Water consumption was either 540 or 2333 L kg_{DW}^{-1} in the scenarios with or without recirculation from harvesting, respectively; N and P supply were 100 g kg_{DW}^{-1} and 16 g kg_{DW}^{-1}, respectively, in the scenarios with added nutrients, while 100 L kg_{DW}^{-1} for manure slurry was considered in other scenarios; the consumption of chemicals was 0.03, 0.08, and 20 g kg_{DW}^{-1} for enzyme, NaOH, and flocculant, respectively; energy consumption was ~1 kWh kg_{DW}^{-1} (optimized plant), while heat for hydrolysis was 1.8 MJ kg_{DW}^{-1}.

Other seven bioreactors installed in the same facility of Almería were assessed by Pechsiri et al. (2023). *Scenedesmus almeriensis* was cultivated to extract biostimulants. Paddlewheel (~31 kg kg^{-1}), LEDs (1.1-15.4 kg kg^{-1}), PVC for electrocoagulation chamber (0.3 kg kg^{-1}), and PMMA for reactor vessel (0.3 kg kg^{-1}) dominated the construction materials in the ORP, indoor PBRs, TLC and VSt, respectively; water consumption was in the range 23-590 kg kg^{-1}, most for cultivation or cleaning in the open or closed systems, respectively; nitrate and phosphate were 100 and 10 g kg^{-1}, respectively; chemicals were ~10-30 g kg^{-1}; electricity was ~11-85 kWh kg^{-1}, while heat for enzymatic hydrolysis was negligible.

This study aims to compile the LCI of an industrial-scale PBR plant installed in Italy, thus taking a further step in the characterization of commercial implementations. The gathered primary data were elaborated, analyzed, and compared with LCIs from recent studies.

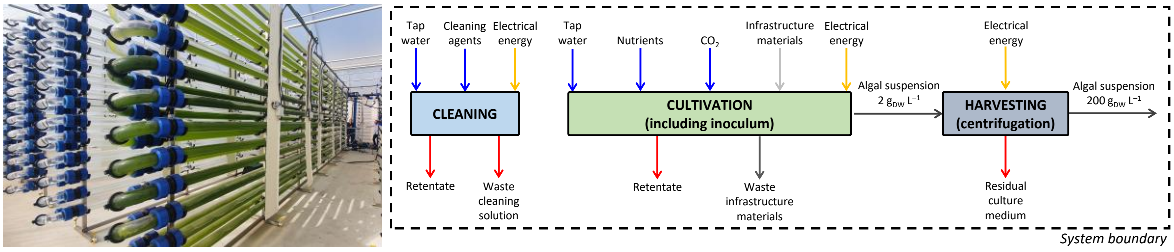
Table 1: Industrial-scale microalgal plants providing primary data for previous LCA studies. Some examples of pilot plants are included. The references marked with "" are used in this paper for LCI comparisons.*

Reference	Reactor type	Reactor size
Passell et al. (2013)	ORP	1000 m^2
Beal et al. (2015)	Horizontal serpentine airlift-driven PBR + ORP	25 + 60 m^3
Silva et al. (2015)	Vertically stacked PBR	12 m^3
Pérez-López et al. (2017)*	Horizontal PBR (Hor), vertically stacked PBR (VSt), ORP	0.56, 1.06, 4.73 m^3
Onorato and Rösch (2020)*	Flat Panel Airlift PBR (FPA), Green Wall Panel PBR (GWP), Unilayer Horizontal Tubular PBR (UHT)	93, 0.1, 93 m^3
Herrera et al. (2021)*	ORP	1000 m^2
Pechsiri et al. (2023)*	ORP, Thin Layer Cascade (TLC), vertically stacked PBR (VSt), Light Exchange Bubble column PBR (LEB), LEB mini reactors MR-1, MR-2, MR-3	20, 3, 4, 0.85, 0.36, 0.36, 0.35 m^3
Bradley et al. (2023)	Horizontal PBR + fermenters	60 + 3 m^3

2. Materials and methods

The inventoried plant is installed in Caltagirone, Sicily (Italy), within a facility occupying an area of ~1500 m^2. *Chlorella vulgaris*, from which valuable products (e.g., biostimulants, lutein, and alternative proteins) can be extracted, is cultivated in vertically stacked (VSt) tubular horizontal PBRs with a total volume of 40.4 m^3 (6 modules × 5.77 m^3 + 2 modules × 2.88 m^3, each module having either 4 or 2 loops of 1442 L) located in an 800 m^2 greenhouse (Figure 1a). The plant capacity is 1200 kg_{DW} $year^{-1}$ and its average productivity is ~0.08 g_{DW} L^{-1} day^{-1}. After cultivation, centrifugation harvesting increases the biomass concentration from 2 to 200 g_{DW} L^{-1}.

The functional unit (FU) chosen to report all elementary input/output flows is 1 kg of dry-weight biomass (which is contained in 5 L of post-harvesting algal suspension). Energy, nutrients, water, chemicals, and infrastructure materials are inventoried to characterize the plant operation and construction. The LCI analysis was carried out by considering average flows representative of one year of industrial plant operation with 300 operating days per year and 10 years of PBR material lifespan. The cradle-to-gate approach was adopted, as depicted in Figure 1b, showing the main flows. The product system was divided into three subsystems, representing the process steps of reactor cleaning, cultivation (including inoculum), and harvesting. Cleaning and cultivation use demineralized water produced by reverse osmosis (RO) of tap water (~700 mg/L TDS) and collected in storage tanks, each of 1500 L. The pre-treatments include quartzite filtration (100 µm), activated carbons, and cartridge microfiltration (10 and 5 µm). Eight RO modules (FILMTEC^{TM} BW30-4040, DuPont) are arranged in four parallel pressure vessels operated at 8-12 bar, producing ~1 m^3 h^{-1} of permeate with ~90% water recovery. Waste streams (red arrows in Figure 1b) are discharged into the sewer.



(a) (b)

Figure 1: Cultivation of Chlorella vulgaris in the industrial-scale plant at the Plastica Alfa facility (Caltagirone, Italy): (a) Photo of the greenhouse with a portion of PBRs; (b) schematics of the analyzed process system.

2.1 Reactor cleaning

The procedure for the PBRs cleaning and sterilization lasts 10 h and encompasses four sub-phases of flushing, each occurring in closed-loop: cleaning with an 80 mg/L sodium troclosene solution (6 h), washing with demi water (1 h), cleaning with a 3% citric acid solution (2 h), washing with demi water (1 h). The PBR modules are cleaned when needed, depending on the process conditions, to restore an optimal environment for microalgae growth. On average, it occurs roughly 5 times per year, thus the process is conducted with an average of 5 complete cycles per year. The exhausted washing solutions are discharged into the sewer.

2.2 Cultivation (including inoculum)

The average growth rate is ~0.15 g_{DW} L^{-1} d^{-1} in both inoculum and cultivation. The concentration target of all growth phases is 2 g_{DW} L^{-1}. The culture medium is prepared with demi water and added nutrients (~8 g/L as total concentration). The RO treatment provides an almost pure water that guarantees conditions suitable for the downstream extraction of high-added value compounds. CO_2 is supplied from pressurized cylinders.

The initial inoculum is performed in multiple steps, starting from growing the biomass in 1 L, then in 10 L, and culminating with an industrial-scale system consisting of 6 vertical airlift PBRs of 90 L each (540 L in total). The target concentration is achieved in ~20 days. UV light is used for sanitization of the fresh medium. The inoculum set-up is provided with (i) a monitoring and control system for the main process parameters, (ii) LED lamps, and (iii) a heat pump thermoregulation system. 500 L of inoculum are used in one loop of the VSt PBRs to start up the cultivation, while the remaining 40 L are used to reactivate the inoculum.

The cultivation in the VSt PBRs is a semi-continuous process. Indeed, a start-up phase takes place through a three-stage dilution (with fresh medium) and growth process after cleaning. Overall, the start-up step lasts ~20 days and the PBRs are filled at ~70% with the liquid. Then, the cultivation batches “at regime” are performed by replacing 500 L of algal suspension per PBR loop with fresh medium, thus growing the microalgal biomass from ~1 to 2 g_{DW} L^{-1} (6.13 days). The cultivation at regime is performed for ~39 batches for each loop per year.

The liquid is circulated through the cultivation PBRs by seven pneumatic pumps (one pump for 4 PBR loops). Compressed air (produced by a compressor and stored in a vessel) is used to drive the pumps and to mix the liquid in the PBRs. Nutrients are dosed in a tank containing the fresh medium (~130 L) that is continuously recirculated during UV sterilization. The culture temperature is maintained at the optimal value of 24 °C with a thermoregulation system based on a heat pump and including a recirculation pump and shell and tube heat exchangers. During some dark hours (~8 h/day) the photosynthetic algal growth is maintained with the support of artificial light provided by LEDs (4 panels per each couple of PBR loops).

2.3 Harvesting

The algal suspension (~1650 L/day) is concentrated via centrifugation. The centrifuge (Macfuge 325) works ~1 hour per day producing a dewatered suspension at 200 g_{DW} L^{-1} (concentration factor equal to 100).

3. Results and discussion

Table 2 reports the LCI results. Overall, more than 1.1 m^3 kg_{DW}^{-1} of tap water is consumed almost in equal portions in the two phases of cleaning and cultivation, producing an almost equal total amount of wastewater (mainly from cleaning and harvesting). The chemical consumption amounts to ~4 kg kg_{DW}^{-1} for both cleaning and cultivation, with major contributions in the two phases due to citric acid and sodium bicarbonate, respectively. PMMA usage in the PBRs is ~0.8 kg kg_{DW}^{-1}. The total energy consumption of the plant amounts to ~376 kWh kg_{DW}^{-1} and is dominated by thermoregulation (217 kWh kg_{DW}^{-1}) and pumping & agitation (115 kWh kg_{DW}^{-1}), followed by LED lighting (40 kWh kg_{DW}^{-1}), in the cultivation phase. In contrast, energy consumption plays a marginal role in cleaning and harvesting (centrifugation). The CO_2 consumption was estimated from laboratory tests, assuming also a mild loss (20% of the input) limited by the dosage controlled by pH measurements. The photosynthetic oxygen was estimated as 1 kgO_2 $kgCO_2^{-1}$ assimilated. Data on possible residual chemicals in the wastewater are not available, but chemical analyses will be performed in the future.

Table 2: Life Cycle Inventory of the analysed full-scale PBRs plant producing Chlorella vulgaris. All values are reported for the FU of 1 kg of DW biomass.

Cleaning			**Cultivation**			**Harvesting**		
Input	*Unit*	*Value*	*Input*	*Unit*	*Value*	*Input*	*Unit*	Value
Tap water	L	563	Tap water	L	556			
Sodium troclosene	g	10	$Na_2MoO_4 \cdot 2H_2O$	g	33			
Citric acid	g	3797	$CaCl_2 \cdot 2H_2O$	g	167			
			$Na_2EDTA \cdot 2H_2O$	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			
Air for pumping&agitation	m^3	22	Air for pumping&agitation	m^3	2672			
			CO_2, kg		2.5			
			PMMA for PBR infrastructure	g	810			
Pumping&Agitation	kWh	2	Pumping&Agitation	kWh	115	Centrifugation	kWh	1.4
			Lighting	kWh	40			
			Thermoregulation	kWh	217			
Output	*Unit*	*Value*	*Output*	*Unit*	*Value*	*Output*	*Unit*	*Value*
Wastewater	L	563	Wastewater	L	56	Wastewater	L	495
Air	m^3	22	Air	m^3	2672			
Residual chemicals	g	N.A.	CO_2	kg	0.5	Residual chemicals	g	N.A.
			O_2	kg	2			
			Waste plastic mat.	g	810			

A comparative analysis of material and energy inputs was performed with LCI results from the literature (Figure 2). From Pérez-López et al. (2017), data referring to the fall season were selected by assuming that they are the most representative of the yearly average behaviour. Figure 2 shows that all inputs are scattered across at least two orders of magnitude and that the plant inventoried in this study has an intermediate performance. The low water consumption of several systems (FPA and GWP from Onorato and Rösch, 2020, all systems from Pechsiri et al. 2023) is due to recirculation from harvesting, while the high water consumption of the UHT system is due to PBR tubes cooling via water spraying. The minimum and maximum values of water consumption from Herrera et al. (2021) fall in the medium-high range, but they considered also a wastewater scenario, whose actual minimum consumption of freshwater or seawater is zero. Regarding the consumption of chemicals, the plant inventoried in the present study is characterized by high values in line with those reported by Pérez-López et al. (2017), while other studies show values reduced even to ~1 g kg_{DW}^{-1} for cleaning and to zero for cultivation (nutrients from wastewater). Moreover, the present plant exhibits a middle-high value of plastic mass for bioreactor construction, in line with other VSt systems. The total energy consumption of the present plant is quite high. Compared to the values reported by other studies (where applicable and available), its consumption for thermoregulation is the lowest one, while its consumption for lighting is the second lowest, likely due to the favorable climatic conditions. However, energy for pumping and aeration plays a major role.

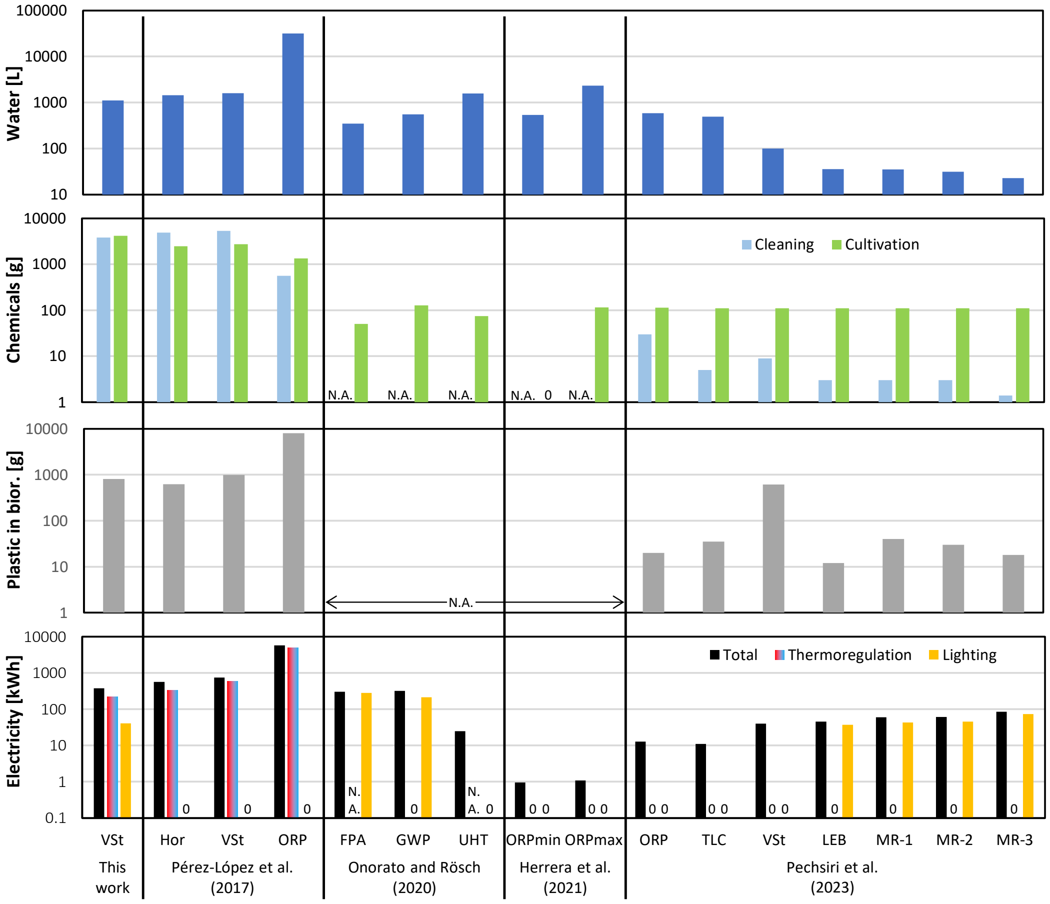


Figure 2: Main inputs (per kg of DW biomass) of pilot/industrial-scale plants for microalgae cultivation.

4. Conclusions

An LCI analysis of an industrial-scale plant cultivating *Chlorella vulgaris* was performed by using primary data. The results revealed that the plant consumes ~1120 L kg_{DW}^{-1} of tap water, 7960 g kg_{DW}^{-1} of chemicals, 810 g kg_{DW}^{-1} of plastic in the PBRs, and 376 kWh kg_{DW}^{-1} of electricity. Water and chemicals are used in similar amounts between the cleaning and cultivation phases, while energy is almost fully consumed in the cultivation phase due to thermoregulation, pumping and aeration, and lighting (217, 115, and 40 kWh kg_{DW}^{-1}, respectively). Compared to other inventoried plants, the present one exhibits intermediate levels of resource consumption. Data from the literature are scattered across several orders of magnitude, affected by technical features and process performance (i.e., productivity), including factors related to the location. Moreover, some LCIs are incomplete and not fully transparent, thus weakening the comparison. However, the results highlight that strategies of water recycling (including the use of wastewater, depending on the final product) and energy optimization are crucial for the minimization of resource consumption. The collected primary data will be integrated with further details, including construction materials and transport of materials, thus providing a robust base to conduct LCA studies of great interest for the environmental analysis of commercial implementations.

Abbreviations

DW – dry weight
FPA – flat panel airlift (PBR)
FU – functional unit
GWP – green wall panel (PBR)
Hor – horizontal (PBR)

LCA – life cycle analysis
LCI – life cycle inventory
LEB – light exchange bubble column (PBR)
LED – light-emitting diode
MR – (LEB) mini reactor
N.A. – not available
ORP – open raceway pond
PBR – photobioreactor
PMMA – polymethylmethacrylate
PP – polypropylene
PVC – polyvinylchloride
RO – reverse osmosis
TLC – thin layer cascade
UHT – unilayer horizontal tubular (PBR)
UV – ultraviolet
VSt – vertically stacked (PBR)

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