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A Comparative Analysis of Environmental Impacts of Operational Phases of Three Selected Microalgal Cultivation Systems

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Abstract: In recent years, microalgal biomass cultivation has been growing in importance, not only related to the production of alternative foods and nutritional supplements but also for its usage for energy purposes or as a natural solution for wastewater treatment. Regarding these cases, the practical potential associated with the circular economy is evident. However, this is not an option for microalgal food and supplements due to strict hygiene requirements for microalgae cultivation used for these purposes. Currently, the most common cultivation options for microalgae include phototrophic cascades, photobioreactors, and heterotrophic fermenters. Generally, the higher requirements for the purity of the resulting biomass, the higher the consumption of energy and nutrients needed. These are the main operational parameters that significantly shape the total environmental and economic performance of microalgae cultivation processes. The comparative Life Cycle Assessment (LCA) of environmental aspects in the operational phases of three selected cultivation systems, located in the Czech Republic and used for pure microalgae biomass production, confirmed that the impacts of these systems in the assessed categories are fundamentally dependent on the amount of electricity needed and nutrient consumption, as well as their sources. For this reason, the heterotrophic fermenter was evaluated as being the most damaging in the comparison of the three cultivation systems, while the phototrophic cascade showed a lower total environmental impact by 15% and the flat photobioreactor was lower still, by 95%, mainly due to energy production from biomass. The major impact categories observed were climate change, depletion of fossil fuels, human toxicity, and freshwater and marine ecotoxicity. The environmental impacts of microalgae cultivation systems could be further reduced if cycling practices, such as process water recycling and reprocessing of generated sewage sludge, were addressed.

Keywords: cascade; photobioreactor; flat-panel; phototrophic; heterotrophic; microalgae; Life Cycle Assessment

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1. Introduction

Compared to traditional crops, microalgae have several advantages. In addition to food, agriculture, and energy, their biomass is widely used in a number of fields [1]. Fertile soil is not necessary for microalgal cultivation; moreover, microalgae are very effective regarding nutrient usage and, thus, in preventing and reducing water pollution by not using fertilizers. In addition, microalgae can be used as soil biofertilizers themselves, as they are capable of using atmospheric nitrogen and have proven to be an interesting alternative for fertilizing various soils contributing to the sustainable fulfillment of the green circular economy [1–3]. Nevertheless, current limitations on the large-scale cultivation of microalgae biomass include several factors, among which the factor associated with high energy consumption is of the greatest importance.

There have been a number of cultivation systems used both on laboratory and industrial scales for microalgal biomass production [4–6]. Generally, the cultivation systems are split into two basic groups: (a) open systems and (b) closed reactors.

Natural ponds or artificial open tanks, raceway ponds, and circulating cascades belong among open microalgae cultivation systems [4,6]. The most common of all open cultivation systems are raceway ponds. They are shallow ponds (between 10 and 50 cm deep, which allow appropriate light intensity) incorporating low energy consumption paddle wheels for gas/liquid mixing and circulation [2,7,8]. The algae culture is directly exposed to the atmosphere, allowing the atmospheric uptake of CO_2 together with the natural use of sunlight, liquid evaporation, and process temperature regulation. Circulating cascades are also commonly used, especially in countries with low insolation intensity or short growing seasons. These systems are constructed as a system of inclined surfaces folded into cascades [6,9,10].

Various reactors, such as closed stirred tanks, airlift reactors, and column, tubular, or panel reactors, which can be operated with horizontal and vertical flow, belong among the closed cultivation microalgal systems [4–7]. In the case of closed systems, the variability of these reactors is very high. In general, their construction and technical advantages depend on whether they are placed in open spaces or intended for rooms. Small compact room reactors could be easily tempered, while large-scale outdoor systems such as tubular photobioreactors need a high technical effort for their tempering [8]. Nevertheless, during the last decades, a great effort has been undertaken to design sophisticated large-scale controlled intensive photobioreactors. These reactors are equipped with double walls, internal illumination, heating and cooling water circulation, etc. [11–14].

Generally, closed systems for microalgae cultivation (photobioreactors) are more energy-intensive and thus energy-demanding than open systems (raceway ponds or cascades). On the contrary, open systems do not offer such a high quality of the resulting product; their operation may be unstable, and keeping a selected strain pure without any contamination for a long time is difficult [15,16]. Both of these factors directly affect the economy of the cultivation process. Concerning microalgal biomass, to be economically competitive and sustainable, supplemental nutrients and operating costs need to be significantly reduced. In particular, the operating costs of mixing [7,17] and heating [15], which are very high in closed reactors, need to be kept at an economically acceptable level in comparison with the amount of dried biomass produced [18].

Heterotrophic cultivations represent a cost-effective, large-scale alternative for some microalgae capable of using organic carbon as their sole carbon and energy source [19]. Such cultivation eliminates the demand for light and offers the possibility of significantly increasing the concentration of cells as well as volume productivity [20]. Controlled growth under defined aseptic conditions reduces nutrient loss and simultaneously increases product quality [4,8]. Photobioreactors and fermenters have many parameters in common: pH and temperature control, harvesting, mixing, degassing, etc. The significant differences between photobioreactors and fermenters are the energy source, oxygen supply, and sterility, as well as some advantages such as a high biomass yield, lower requirements for light, and easy control of monocultures. In heterotrophic cultivation, microalgae cultures utilize some organic compounds (e.g., glucose or acetate) as a source of carbon and energy for growth [6]. Using wastewater as a nutrient source could also be an attractive option, but only for non-food products [2,21]. Relatedly, it must be emphasized that the preparation of high-value microalgal products aimed to be used in food, pharmaceutical, and cosmetic industries is only feasible in closed photobioreactors with a high multiplication capacity for cultivation conditions [6,11], which must follow systematic principles of the Good Manufacturing Practice (GMP) and of the Hazard Analysis and Critical Control Points (HACCP).

Haematococcus pluvialis, *Chlorella vulgaris*, *Chlorella pyrenoidosa*, *Dunaliella salina*, *Schizochytrium* sp., *Scenedesmus* sp., *Muriellopsis* sp., *Porphyridium* sp., *Rhodella* sp., etc., belong among algae species cultivated in food and nutritional supplement's industries [4,22–25]. These species of green and red algae are often used to produce carotenoids, chlorophylls, phycobilins, and other natural dyes. Green and brown microalgae, such as *Dunaliella salina*, *Phaeodactylum tricornotum*, *Pleurochrysis carterae*, *Chlorella* sp., *Chlorococcum* sp., *Nannochloropsis* sp. or any other green algae of Chlorophyta, are used in biofuel and fertilizer industries [1–3,7,26,27].

The aim of this work was to perform a comparative Life Cycle Assessment (LCA) of the operational phases of three selected technological methods for microalgae cultivation (i.e., phototropic cascades, a heterotrophic fermenter, and a flat-panel photobioreactor), including basic processing of produced algal biomass. Since algae cultivation has a long tradition in the Czech Republic (e.g., Masojidek and Torzillo [6]; Binova [9]), the authors decided to analyze the technological facilities available there, which they could visit and observe in 2019–2020. Considering the potential sensitivity of the information provided, only the information that was further verified as publicly available was used for the assessment. Overall, the main objective of this study was to identify bottlenecks in the compared microalgae cultivation systems and to point out possibilities for minimizing their significant impacts. Last but not least, the authors wanted to point out that even simple technological systems have a chance to be interesting from an environmental point of view.

2. Description of Assessed Cultivation Systems

2.1. Phototrophic Cascade

Within the Czech Republic, microalgae have been grown phototrophically in Trebon, in the South Bohemian region, since 1962. The cultivation takes place in an area of 900 m^2. The cultivation device consists of a steel structure that supports glass panels. The cultivation system is based on the use of inclined surfaces, along which the algae suspension permanently flows in a thin layer (3 to 5 mm). The algae suspension flows at the optimum speed, which is determined by the slope of the surface and under optimal turbulence. An inorganic nutrient solution is saturated with CO_2 and, owing to the energy of sunlight, algae cultures multiply and grow. Under the glass panels, collection tanks, into which algae is drained from the glass cascades, are placed. Figure 1 shows a schematic drawing and an accompanying photo of the cascade system. A detailed description of this system is available in the CZ patent [28] or in the utility model [29].

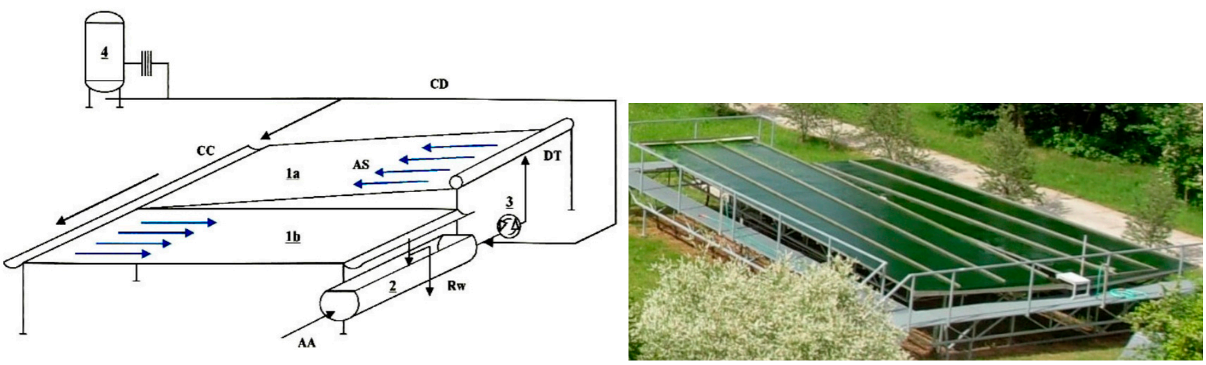

Figure 1. The phototrophic cultivation cascade—a schematic drawing and an overall photo [9,30]. legend: 1 a,b—cultivation areas; 2—a collection tank; 3—a circulation pump; 4—a carbon dioxide reservoir; AA—algae suspension generation; AS—algae suspension flow; CC—a connecting trough; CD—a distribution pipe; DT—a transition trough; Rw—rainwater and washing water.

The harvesting is carried out when the weight of algae biomass in 1 L of the nutrient solution reaches about 30 g. The device is advantageous mainly in terms of the quality of the grown algae. In the climatic conditions of South Bohemia (the Czech Republic), the biomass yields of *Chlorella* sp. algae per area of 1 ha range from 25 to 30 tons of dry algae biomass per one cultivation season, which lasts approximately 150 days [9].

During harvesting, a disc centrifuge separates the algal biomass from water containing residual nutrients; subsequently, the biomass is repeatedly rinsed with clean wash water to remove the residual nutrient solution. The next step includes a disintegration and mechanical dissolution of algal cellulose cell walls. In this technological step, the disintegration of algal biomass cells higher than 85% is achieved on average. The final step is gentle drying carried out in a spray dryer, where the pulpy mass of sprayed biomass is dispersed with a maximal speed of 30 L/h [31] and immediately dried at a temperature not exceeding 60 °C [9]. The direction of spray drying is downward co-current; the temperature of the inlet and outlet air varies between 55–60 °C; drying time is approx. 1.5 s.

2.2. Heterotrophic Fermenter

A heterotrophic cultivation fermenter based in Trebon, South Bohemia, the Czech Republic, was assessed as the second-best algae cultivation system. The heterotrophic method of cultivation allows for high growth rates and harvest concentrations of over 100 g/L to be achieved within a few tens of hours versus units of grams of autotrophic cultivation. In addition, heterotrophic cultivation can be carried out throughout the whole year when the sunlight intensity is insufficient. Glucose ($C_6H_{12}O_6$, CAS: 55-99-7) is used for the heterotrophic cultivation of microalgae there [9].

Details on heterotrophic cultivation performed on glucose are given in the CZ patent [32]. Figure 2 shows a photo of the assessed fermenter with an operating volume of 150 L [31]. The fermenter is standardly equipped with a feeding pump for nutrients and water, mixing, cooling/heating, aeration, a steam unit for sterilization and cleaning, and an outlet for the discharge of washing water and algae harvesting.

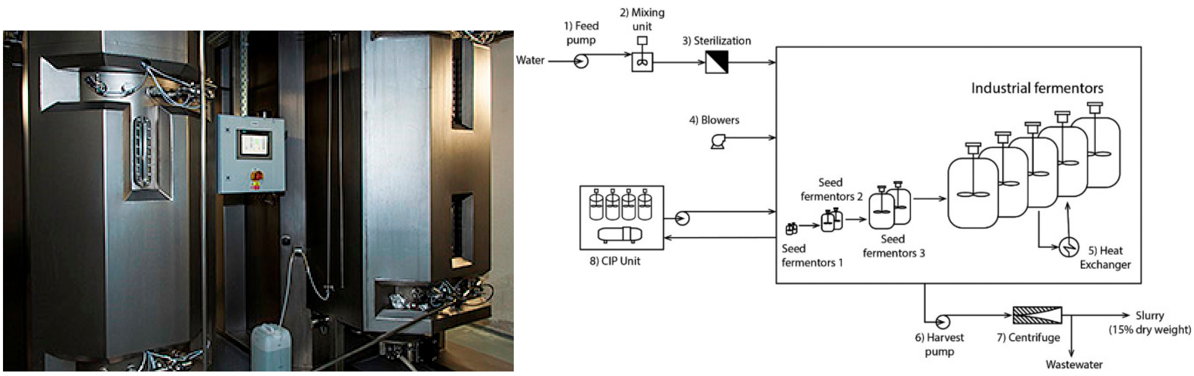

Figure 2. The heterotrophic fermenter—an overall photo and a typical scheme of the heterotrophic production [31,33]. Notes: CIP—cleaning in place.

The process of harvesting algae biomass after heterotrophic cultivation is the same as in the case of phototrophic cascade cultivation mentioned in Section 2.1.

2.3. Flat Panel Airlift Photobioreactor

Large-scale algal biomass cultivation in a flat panel system is carried out in Mostek, which is a small mountain village located in the East Bohemian part of the Czech Republic. The facility is designed as a full indoor system using Subitec Flat Panel Airlift (FPA) production photobioreactors combined with line and automatic modules, which ensures the maximum output per volume with high and stable product quality [34]. The production site was built in 2014–2015; its operation was started in 2016. The company specializes in the cultivation and subsequent extraction of the active substances of astaxanthin. Figure 3 shows the *Haematococcus pluvialis* cultivation scheme used and a photograph of the factory premises.

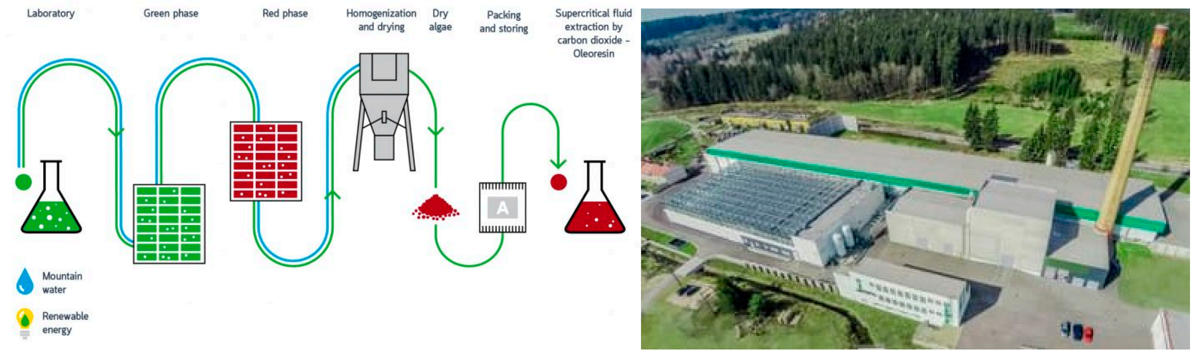

Figure 3. The FPA photobioreactors—a production scheme of astaxanthin and a photo of the production facility premises [34].

The cultivation itself begins under laboratory conditions and continues through laboratory photobioreactors and micromodules to mass production in FPA photobioreactors. The entire process is completely system-controlled and operates according to HACCP principles. In addition, using fully green energy and heat from the neighboring biomass power plant and recycling the cultivation water by reusing it in the production process can be considered sustainable and environmentally friendly [35]. The production facility itself operates with a volume of 130,000 L and consists of 700 FPA photobioreactors equipped with artificial lighting (LED—light emitting diode, intensity 200 W/m^3). The total area of the production is 4000 m^2 [36]. Oleoresin is obtained from algae cells by supercritical CO_2 extraction [35]. In terms of biomass production, yields of *Haematococcus pluvialis* grown in the FPA panels averaged 7–12.3 g/L, while astaxanthin concentrations ranged between 114 and 602 mg/L [37,38].

3. Materials and Methods

3.1. Study Objective and System Boundaries

The LCA study was carried out in accordance with the latest amendments of the standards of ISO 14040:2006 [39] and ISO 14044:2006 [40]. All process parameters used were chosen to allow a comparison of the selected technological configurations of microalgae cultivation systems.

The technical boundaries of the LCA study were limited to the operational phase of the assessed technological arrangements of microalgae cultivation. Resource materials, construction work, and building of the production units, as well as their disposal and related transporting of equipment to a production site, were not included. On the other hand, the processes related to obtaining raw materials (water), the production of chemicals (nutrients), and energy (electricity, steam), together with the processes related to managing waste (wastewater, waste biomass), were included in this study. Given that all assessed cultivation units are located in the Czech Republic, and all supplementary materials originate from the same country, it can be stated that the geographical boundaries of the study were limited to the Czechia territory. The environmental boundaries of this comparative study are determined by elementary flows (emissions) from the operational phase to the soil, water, and air. The material intensity of the assessed systems was considered by including all raw material flows involved in the production of the necessary operating substances and energy. Cut-off criteria were not considered during the presented study. The allocation of the processes was only physical; economic or causal allocations were not applied.

As part of this LCA study, it was necessary to apply the following assumptions and simplifications:

1. The algae biomass was assessed as a homogeneous material in the study.
2. Neither the content of active substances in algal biomass nor the type of cultivated microalgae was taken into account.

3. The source of nutrients for algae cultivation was represented by its main components (two in the case of the phototrophic cascade and the heterotrophic fermenter and three for the flat panel photobioreactor).
4. Wastewater produced was considered to be waste; no treatment and its reuse were considered.
5. Waste sludge from algae production was considered waste without any further downstream recovery.

3.2. Functional Unit and Performance Parameters

A functional unit (FU) represents a quantitative function description of the studied system [41]. It plays a significant role in LCA and can influence the comparative performance of assessed systems [42], as FU, under this assessment, serves as a benchmark for comparing assessed systems or technological variants. Therefore, the operational parameters of three assessed technological arrangements of microalgae cultivation were recalculated to the set FU so that these configurations could be compared with each other.

The final product considered in this LCA assessment was all process-dried biomass (FU = 1 kg of dried biomass). Waste products of cultivation consisted of wastewater (87–89%), sewage sludge (3–5%), and evaporation or operating losses (6–10%). The subsequent technology for processing dry algae biomass was no longer assessed within the LCA. The aggregate input values of material and energy flows of the individual technologies compared in this study are presented below. The following inputs were included:

- Phototrophic cascade (PC): drinking water (a commercial water supplier) 1800 L; energy (mix CZ) 1450 kWh; nutrients (commercial chemicals) 1.6 kg and CO_2 (a commercial gas) 11 L.
- Heterotrophic fermenter (HF): drinking water (a commercial water supplier) 4450 L; energy (mix CZ) 2180 kWh and nutrients (commercial chemicals) 70 kg.
- Flat-panel photobioreactor (PBR): drinking water (a groundwater collection) 160,000 L; energy (biomass) 32 MWh; nutrients (commercial chemicals) 2.5 kg and CO_2 (a commercial gas) 3200 L.

According to the comparative study [43], the technological operation of the assessed size arrangements of microalgae cultivation was recalculated to the determined FU (i.e., 1 kg of dry algae biomass). Details are given in Table 1.

Table 1. Operational parameters of the assessed microalgae cultivation systems converted to the set FU (1 kg of dry algae biomass).

Inputs	Unit	PC	HF	PBR
Water	L	93.5	164.8	205.1
Energy	kWh	75.3	80.7	41.0
Nutrients	kg	0.08	2.59	0.01
CO_2	kg	0.43	-	3.0
Outputs	**Unit**	**PC**	**HF**	**PBR**
Dried algae biomass (FU)	kg	1.0	1.0	1.0
Wastewater (incl. operation. losses)	L	81.4	143.3	178.2
Waste sludge	L	2.8	4.9	10.0
Evaporation (water vapor)	kg	0.006	0.005	0.104

Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor, FU—functional unit.

3.3. LCA Software and Impact Assessment Method

The comparative study was modeled in the openLCA software v.1.10.3 (GreenDelta, Berlin, Germany, 2020) with the ecoinvent v.3.6 database use (Ecoinvent, Zürich, Switzerland, 2019). Based on its nature, it was decided to use an APOS_unit model, which follows an attribution approach, where loads are proportionally assigned to specific processes.

The resulting impacts were assessed using the CML baseline method (one of the frequently used Life Cycle Impact Assessment methods) created by the Institute of Environmental Sciences, University of Leiden (Leiden, The Netherlands) in 2001 [44]. This assessment method includes a major group of midpoint impact categories such as source depletion, climate change, toxicity to humans, and water and terrestrial ecotoxicity [45]. The used CML baseline method v.4.4 of January 2015 was provided within the 'openLCA software as the LCIA methods package v.2.1.2 (GreenDelta, Berlin, Germany, 2021) and was compatible to be used with the ecoinvent v.3.6 database.

4. Results

4.1. Inventory Analysis

Table 2 shows the summary results of the Life Cycle Inventory (LCI) for selected impacts for three compared technological methods of microalgae cultivation systems. Overall statistics showed for the cultivation systems between 14,694 to 14,695 related processes and between 360,502 to 360,520 related process links. This statistical information applies to the software and database used, as generic data from the ecoinvent database were also used. The number of processes is linked to the process with the highest in-degree (linked inputs) and highest out-degree (linked outputs). The process links are related to the number of input and output processes with selected default providers. In this study, the processes related to the supply of necessary electricity and nutrients, together with the disposal of the generated waste, were represented by the highest numbers, which corresponded to the relevant impact categories, such as fossil fuel consumption, climate change, toxicity to humans, freshwater and marine ecotoxicity.

Table 2. Results of inventory analysis (LCI) of the compared cultivation systems.

Environmental Impacts	Unit	PC	HF	PBR
Acidification potential	kg SO_2 eq.	0.243	0.285	0.007
Climate change	kg CO_2 eq.	69.014	72.849	2.019
Depletion of elements	kg Sb. eq.	1.4×10^{-6}	1.3×10^{-6}	1.8×10^{-7}
Depletion of fossil fuels	MJ	604.26	683.68	20.521
Eutrophication	kg PO_4 eq.	0.366	0.404	0.004
Freshwater ecotoxicity	kg 1,4-DCB eq.	60.261	67.443	1.753
Human toxicity	kg 1,4-DCB eq.	41.126	48.700	5.678
Marine ecotoxicity	kg 1,4-DCB eq.	160.8×10^3	176.5×10^3	29.8×10^2
Ozone depletion	kg CFC-11 eq.	3.6×10^{-6}	4.2×10^{-6}	1.7×10^{-7}
Photochemical oxidation	kg ethylene eq.	0.009	0.010	0.001
Terrestrial ecotoxicity	kg 1,4-DCB eq.	0.113	0.236	0.024

Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor.

4.2. Impact Assessment

Table 3 below gives the weighted results of each indicator for individual impact categories after applying the CML baseline method, which is a frequently used method in LCA [45]. Furthermore, the CML assessment method pays attention to the impacts on human and ecosystem health [46], which were considered to be of the same priority for the performed study as the impacts in the form of global warming and fossil resources depletion. The minimal differences between the inventory data (Table 2) and the data assessed using the basic CML method (Table 3) are due to the nature of the study, as none of the normalization or weighting sets provided with the CML baseline method were used.

Table 3. Assessed results of the inventory of the compared microalgae cultivation systems by the CML baseline method (midpoint).

Assessed Impact Categories (CML Baseline, v.4.4, 2015)	Unit	PC	HF	PBR
Acidification potential (average Europe)	kg SO_2 eq.	2.426×10^{-1}	2.851×10^{-1}	0.658×10^{-2}
Climate change (GWP100)	kg CO_2 eq.	6.901×10^{1}	7.285×10^{1}	0.202×10^{1}
Depletion of abiotic resources (elements)	kg Sb. eq.	1.436×10^{-6}	1.332×10^{-6}	0.176×10^{-6}
Depletion of abiotic resources (fossil fuels)	MJ	6.042×10^{2}	6.836×10^{2}	2.037×10^{1}
Eutrophication (generic)	kg PO_4 eq.	3.657×10^{-1}	4.043×10^{-1}	0.352×10^{-2}
Freshwater ecotoxicity (FAETP inf)	kg 1,4-DCB eq.	6.026×10^{1}	6.744×10^{1}	0.175×10^{1}
Human toxicity (HTP inf)	kg 1,4-DCB eq.	4.112×10^{1}	4.869×10^{1}	0.567×10^{1}
Marine ecotoxicity (MAETP inf)	kg 1,4-DCB eq.	1.608×10^{5}	1.765×10^{5}	2.973×10^{3}
Ozone depletion (ODP steady state)	kg CFC-11 eq.	3.600×10^{-6}	4.185×10^{-6}	0.171×10^{-6}
Photochemical oxidation (high NOx)	kg ethylene eq.	9.286×10^{-3}	1.046×10^{-2}	0.503×10^{-3}
Terrestrial ecotoxicity (TETP inf)	kg 1,4-DCB eq.	1.133×10^{-1}	2.355×10^{-1}	0.241×10^{-1}

Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor.

Figures 4 and 5 show the most significant impact categories and their differences between the individual reactors compared. The results are similar to the inventory analysis, where a different energy source led to lower impacts on fossil fuel depletion, and thus, climate impacts and toxicity for both humans and water ecosystems when cultivated in a photobioreactor arrangement, whereas higher consumption of nutrients used within the heterotrophic microalgae cultivation increased them.

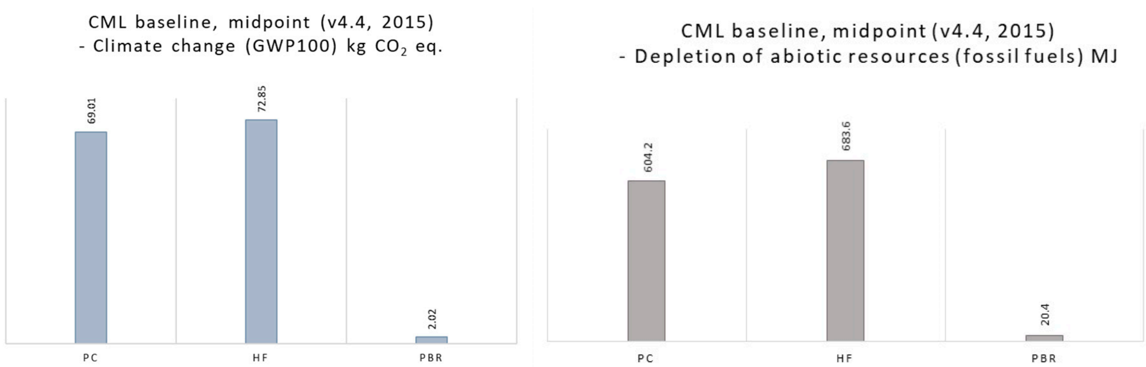

Figure 4. Resulting impacts of compared microalgae cultivation systems (CML baseline, midpoint, v.4.4, 2015)—climate change (**left**), depletion of fossil fuel consumption (**right**). Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor.

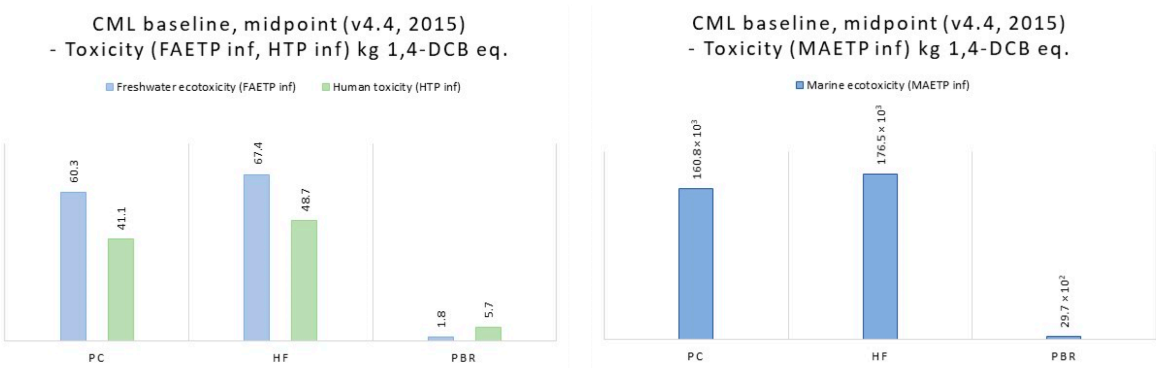

Figure 5. Resulting impacts of compared microalgae cultivation systems (CML baseline, midpoint, v.4.4, 2015)—freshwater and human toxicity (**left**), marine toxicity (**right**). Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor.

Figure 6 shows the relative indicator results of the assessed systems of microalgae cultivations. The maximum result of each indicator is set to 100%, while the results of the other variants are displayed in relation to this result.

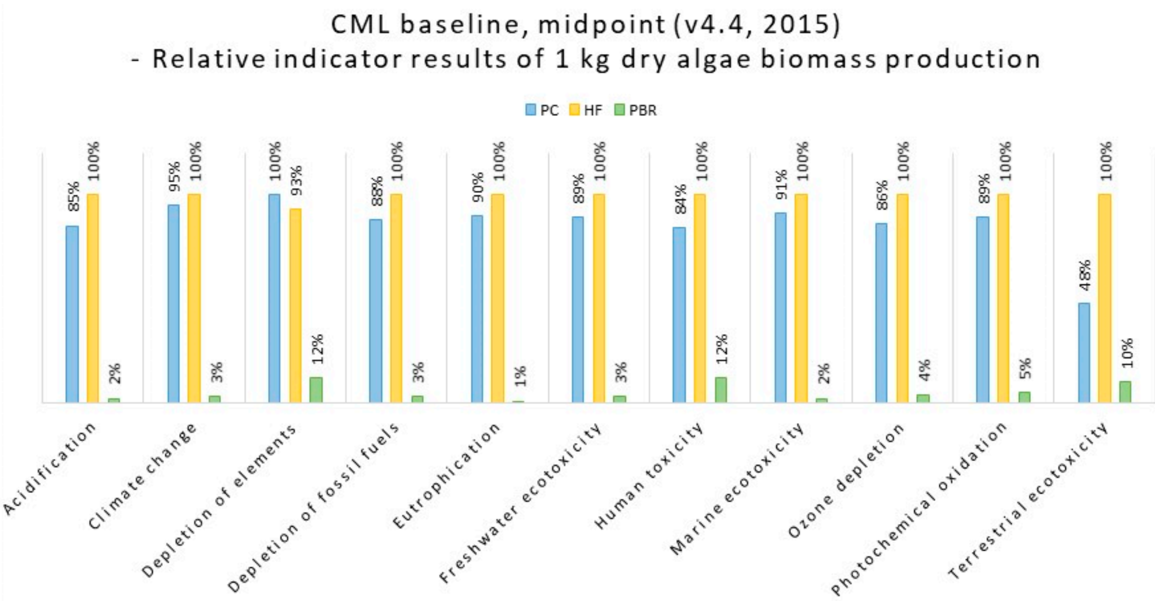

Figure 6. Relative indicator results of the production of 1 kg dry algae biomass (CML baseline, midpoint, v.4.4, 2015). Notes: PC—phototrophic cascade, HF—heterotrophic fermenter, PBR—flat-panel photobioreactor.

4.3. LCA Interpretation and Study Verification

The significant findings of this comparative LCA study, based on the data shown in the previous two sub-chapters, are the following:

- The microalgae cultivation systems under consideration generate environmental impacts, particularly in terms of fossil fuel consumption, climate change, and toxicity to human and water environment (both freshwater and marine).
- The most important factors influencing the level of overall environmental impacts of the assessed cultivation systems are the electricity source, the nutrients used, and the amount of their consumption.

The effect of the energy source was further elaborated in the sensitivity analysis. The analysis of sensitivity was carried out for the flat-panel photobioreactor, which showed the lowest impact from the compared microalgae cultivation systems. During the sensitivity analysis, two energy sources (biomass and CZ energy mix) were considered. Table 4 and

Figure 7 below provide details for the selected impact categories (climate change, fossil fuel consumption, human toxicity, and water ecotoxicity).

Table 4. Sensitivity of the most contributing impacts of the flat-panel photobioreactor on using two different energy sources (biomass, energy mix).

Assessed Impact Categories (CML Baseline, v.4.4, 2015)	Unit	PBR—B	PBR—E
Climate change (GWP100)	kg CO_2 eq.	0.202×10^1	3.936×10^1
Depletion of abiotic resources (fossil fuels)	MJ	2.037×10^1	34.68×10^1
Freshwater ecotoxicity (FAETP inf)	kg 1,4-DCB eq.	0.175×10^1	3.428×10^1
Human toxicity (HTP inf)	kg 1,4-DCB eq.	0.567×10^1	2.744×10^1
Marine ecotoxicity (MAETP inf)	kg 1,4-DCB eq.	2.973×10^3	90.05×10^3

Notes: PBR—flat-panel photobioreactor, B—energy from biomass, E—energy mix.

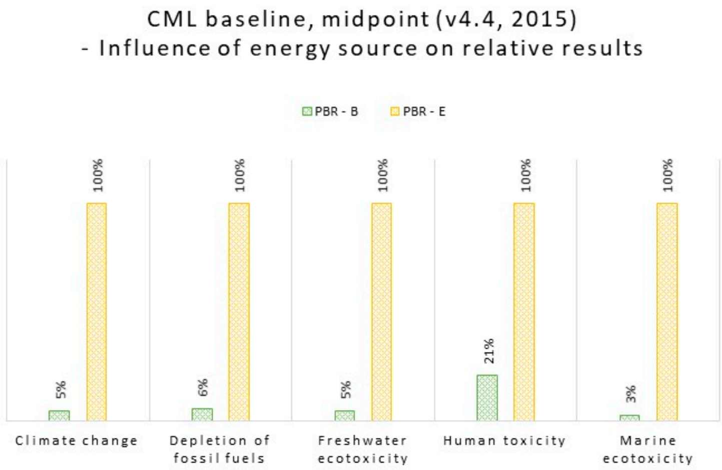

Figure 7. Relative indicator results of 1 kg of dry algae biomass connected with the production in the flat-panel photobioreactor using two different energy sources (CML baseline, midpoint, v.4.4, 2015). Notes: PBR—flat-panel photobioreactor, B—energy from biomass, E—energy mix.

5. Discussion

The comparative study was aimed at identifying the bottlenecks of the compared microalgae cultivation systems and pointing out possible ways to minimize their significant impact categories. Based on the achieved relative results of the comparison study, it is evident that the heterotrophic fermenter showed the highest relative impacts in most assessed environmental impact categories (93–100%), followed by the phototrophic cascade (48–100%) and finally by the flat-panel photobioreactor (1–12%). This finding is clearly related to the energy source since, in the case of the photobioreactor, energy was provided by the biomass combustion facility. Furthermore, this fact was fully confirmed by the performed sensitivity analysis. Changing the supply of green energy to a conventional energy mix caused a significant increase in the major impacts of the flat-panel photobioreactor (by 30 times). However, the operation of this type of photobioreactor still generated half of the total impacts compared to the heterotrophic fermenter and two-thirds compared to the phototrophic cascade.

On the contrary, the consumption of nutrients directly corresponded to the character of individual microalgae cultivation methods [6]. This means that the nutrient consumption was significantly reflected in the level of impact only in the case of the heterotrophic reactor, which relies on nutrients as the sole source of carbon and energy [33]. Surprisingly, the need for carbon dioxide supply in the case of the other two cultivation methods and the greater consumption of process water in the flat-panel photobioreactor did not play as a significant role in the overall environmental impact of the compared cultivation systems as the large nutrient requirement in the heterotrophic cultivation.

The results of the sensitivity analysis showed that green resources of energy influence the overall impact on the microalgae cultivation systems most significantly. It appears reasonable to assume that an introduction of a system for recycling produced wastewater would also contribute to lowering the environmental impacts of all assessed cultivation systems. This could be crucial, especially in the case of the heterotrophic fermenter, where the second largest volume of wastewater is produced per 1 kg of dried biomass. Similarly, it could be applied to the waste sludge produced. Incorporating the circular use of wastewater and sewage sludge in the LCA model could even reduce the total environmental impacts of the flat-panel photobioreactor, which in both cases, produces the largest amount of such waste.

Finally, to obey the principles of the circular economy concept, the use of waste nutrient sources would help to reduce the overall environmental impact of the heterotrophic system while keeping the comparable quality of the final product [21]. Of course, this option must be deeply considered related to the cultivation for human consumption due to strict hygiene standards. However, it should be noted as considerable due to the huge potential of biowaste and current advances in re-processing techniques. Such as hydrolysate produced from waste feathers [47] or biofertilizers produced from wastewater [2], which could serve as a high-quality and sustainable source of nutrients.

Considering in general, the impact categories observed for the microalgal cultivation systems showed major effects on the following five categories—climate change, fossil fuel depletion, human toxicity, and marine and freshwater ecotoxicity. The difference between the individual impact categories was proportional between the assessed systems, i.e., they all showed a similar number of differences between them, regardless of the evaluated impact category. In terms of impact level, an order of magnitude higher impact was modeled for the category of marine ecotoxicity, where their values were at 10^5 values compared to the other major impacts, where their levels were in the range of 10^1. Such a high level of this impact category, as well as in the categories of freshwater ecotoxicity and human toxicity, is caused by the taken assumption of the disposal of the generated waste products (i.e., wastewater and water sludge).

Since the impact categories of toxicological and ecotoxicological effects have been considered problematic for several scientific reasons [41], and therefore it is appropriate to focus on the remaining two impact categories (climate change and fossil fuel consumption), which are directly related to energy and nutrients supplies, as discussed above. Last but not least, it should be pointed out that even a simple microalgae cultivation system, such as the phototrophic cascade, has a chance to be interesting from the point of view of environmental impact assessment, taking into account the lower impacts (on average by 10%) in the most evaluated categories except depletion of abiotic elements and terrestrial ecotoxicity.

6. Conclusions

The study focuses on a comparative environmental LCA of three selected types of cultivation systems of microalgal biomass—the phototrophic cascade (PC), a heterotrophic fermenter (HF), and a flat-panel photobioreactor (PBR). The operational parameters of all assessed systems were based on the systems that are located in the Czech Republic. The LCA comparative model was set as the model for the production of microalgal biomass for human purposes, thus obeying, in general, the strict hygienic requirements. For this reason, the potential use of waste products (e.g., as a nutrient and water resources) for lowering the environmental impacts of the compared cultivation systems and simultaneously supporting the circularity concept was not modeled.

The results of the LCA study showed that the total midpoint impacts of the assessed technological arrangements on the environment are directly related to the source of energy (the conventional electricity mix versus the biomass source) and the need for nutrients used, as well as their overall consumption. The lowest total impact was calculated for the flat-panel photobioreactor (PBR) in all observed impact categories, followed by the phototrophic cascade (PC), which showed a better performance than the last positioned heterotrophic fermenter (HF) in most impact categories. The heterotrophic fermenter (HF) was defined as the most negative option from an environmental point of view. This is mostly due to the high consumption of nutrients related to the system itself.

Climate change, fossil fuel consumption, and human and water toxicity represented the highest impact categories observed; mostly in tens to thousands of related impact units (except for marine ecotoxicity, where it was hundreds of thousands of units). The high values of marine ecotoxicity and other toxicity categories (freshwater and human) are related to the linear concept of the model, where recycling of wastewater and secondary sludge evaporation was not considered. To conclude, with regard to decreasing the impacts of microalgae cultivation for human purposes and also for other purposes such as energy use, it is important to use a targeted combination of available types of green energy sources (e.g., photovoltaics, geothermal energy), cheaper alternative nutrients (e.g., hydrolysate) as well as to cycle processing water and to valorize generated sewage sludge. This suggestion applies to all three compared technological arrangements of microalgae cultivation and is also generally applicable to most cultivation systems of microalgae biomass.

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References

1. Deepica, P.; MubarakAli, D. Production and assessment of microalgal liquid fertilizer for the enhanced growth of four crop plants. *Biocatal. Agric. Biotechnol.* **2020**, *28*, 101701. [CrossRef]
2. de Paula Pereira, A.S.A.; de Siqueira Castro, J.; Ribeiro, V.J.; Calijuri, M.L. Organomineral fertilizers pastilles from microalgae grown in wastewater: Ammonia volatilization and plant growth. *Sci. Total Environ.* **2021**, *779*, 146205. [CrossRef]
3. Ferreira Lorentz, J.; Calijuri, M.L.; Peixoto Assemany, P.; Sousa Alves, W.; Gomes Pereira, O. Microalgal biomass as a biofertilizer for pasture cultivation: Plant productivity and chemical composition. *J. Clean. Prod.* **2020**, *276*, 124130. [CrossRef]
4. Borowitzka, M.A. Commercial production of microalgae: Ponds, tanks, tubes and fermenters. *J. Biotechnol.* **1999**, *70*, 313–321. [CrossRef]
5. Eriksen, N.T. The technology of microalgal culturing. *Biotechnol. Lett.* **2008**, *30*, 1525–1536. [CrossRef]
6. Masojidek, J.; Torzillo, G. Mass cultivation of freshwater microalgae. In *Encyclopedia of Ecology*; Jørgensen, S.E., Fath, B.D., Elsevier, B.V., Eds.; Elsevier: Oxford, UK, 2008; pp. 2226–2235, ISBN 978-0-08-045405-4.
7. Jorquera, O.; Kiperstok, A.; Sales, E.A.; Embiruçu, M.; Ghirardi, M.L. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. *Bioresour. Technol.* **2010**, *101*, 1406–1413. [CrossRef]
8. Ugwu, C.U.; Aoyagi, H.; Uchiyama, H. Photobioreactors for mass cultivation of algae. *Bioresour. Technol.* **2008**, *99*, 4021–4028. [CrossRef]

9. Binova, A. Cultivation, Processing and Food Utilization of Green Algae of the Genus *Chlorella* in the Trebon Region. Bachelor's Thesis, Palacky University Olomouc, Olomouc, Czech Republic, 2014; p. 44. Available online: https://theses.cz/id/z860a6/ (accessed on 8 March 2022).
10. Giannelli, L.; Watanabe, C.; Yamaji, H.; Katsuda, T. Effects of fluid-flow on *Chlorella sorokiniana* cultivation in cascade photobioreactors with either flat or wavy bottoms. *J. Biotechnol.* **2022**, *359*, 15–20. [CrossRef]
11. Pulz, O. Photobioreactors: Production systems for phototrophic microorganisms. *Appl. Microbiol. Biotechnol.* **2021**, *57*, 287–293.
12. Gupta, P.L.; Lee, S.-M.; Choi, H.-J. A mini review. Photobioreactors for large scale algal cultivation. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1409–1417. [CrossRef]
13. Slegers, P.M.; van Beveren, P.J.M.; Wijffels, R.H.; van Straten, G.; van Boxtel, A.J.B. Scenario analysis of large scale algae production in tubular photobioreactors. *Appl. Energy* **2013**, *105*, 395–406. [CrossRef]
14. Subitech. Product Cultivation Module CM118. ©2022a. Available online: https://www.subitec.com/en/industry/cultivation-module/ (accessed on 31 September 2022).
15. Zittelli, G.C.; Biondi, N.; Rodolfi, L.; Tredici, M. Photobioreactors for mass production of microalgae. In *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 2nd ed.; Richmond, A., Hu, Q., Eds.; John Wiley & Sons: New York, NY, USA, 2013; pp. 225–266. ISBN 978-0-470-67389-8.
16. Lam, T.P.; Lee, T.-M.; Chen, C.-Y.; Cang, J.-S. Strategies to control biological contaminants during microalgal cultivation in open ponds. *Bioresour. Technol.* **2018**, *252*, 180–187. [CrossRef] [PubMed]
17. Biondi, N.; Bassi, N.; Zittelli, G.C.; De Faveri, D.; Giovannini, A.; Rodolfi, L.; Allevi, C.; Macrì, C.; Tredici, M.R. *Nannochloropsis* sp. F&M-M24: Oil production, effect of mixing on productivity and growth in an industrial wastewater. *Environ. Prog. Sustain. Energy* **2013**, *32*, 846–853.
18. Sleges, P.M. Scenario Studies for Algae Production. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2014; p. 224. Available online: https://edepot.wur.nl/294573 (accessed on 12 April 2022).
19. Handler, R.M.; Canter, C.E.; Kalnes, T.N.; Lupton, F.S.; Kholiqov, O.; Shonnard, D.R.; Blowers, P. Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts. *Algal Res.* **2012**, *1*, 83–92. [CrossRef]
20. Zhang, C.-D.; Li, W.; Shi, Y.-H.; Li, Y.-G.; Huang, J.-K.; Li, H.-X. A new technology of CO_2 supplementary for microalgae cultivation on large scale—A spraying absorption tower coupled with an outdoor open runway pond. *Bioresour. Technol.* **2016**, *209*, 351–359. [CrossRef]
21. Wu, Y.-H.; Hu, H.-Y.; Yu, Y.; Zhang, T.-Y.; Zhu, S.-F.; Zhuang, L.-L.; Zhang, X.; Lu, Y. Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review. *Renew. Sustain. Energy Rev.* **2014**, *33*, 675–688. [CrossRef]
22. Kopecky, J.; Lhotsky, R.; Paichlova, J. *Active Substances of Microalgae in Nutrition (in Czech Only)*; Ed. no. 12159; Center of Joint Activities of the Academy of Sciences of the Czech Republic: Prague, Czech Republic, 2017; p. 28. ISSN 2464-6245.
23. Doucha, J.; Livansky, K. Production of high-density *Chlorella* culture grown in fermenters. *J. Appl. Phycol.* **2012**, *24*, 35–43. [CrossRef]
24. Slocombe, S.P.; Huete-Ortega, M.; Kapoore, R.V.; Okurowska, K.; Mair, A.; Day, J.G.; Stanley, M.S.; Vaidyanathan, S. Enabling large-scale production of algal oil in continuous output mode. *iScience* **2021**, *24*, 102743. [CrossRef]
25. Tan, X.-B.; Wan, X.-P.; Yang, L.-B.; Wang, X.; Meng, J.; Jiang, M.-J.; Pi, H.-J. Nutrients recycling and biomass production from *Chlorella pyrenoidosa* culture using anaerobic food processing wastewater in a pilot-scale tubular photobioreactor. *Chemosphere* **2021**, *270*, 129459. [CrossRef]
26. Simonazzi, M.; Pezzolesi, L.; Guerrini, F.; Vanucci, S.; Samorì, C.; Pistocchi, R. Use of waste carbon dioxide and pre-treated liquid digestate from biogas process for *Phaeodactylum tricornutum* cultivation in photobioreactors and open ponds. *Bioresour. Technol.* **2019**, *292*, 121921. [CrossRef]
27. Lan Chi, N.T.; Mathimani, T.; Manigandan, S.; Shanmugam, S.; Thi Ha, N.; Cam Nhung, T.; Alharbi, S.A.; Chinnathambi, A.; Brindhadevi, K.; Chanasut, U.; et al. Small scale photobioreactor, outdoor open pond cultivation of *Chlorella* sp. and harvesting at log and stationary growth phase towards lipids and methyl ester production. *Fuel* **2022**, *319*, 123813. [CrossRef]
28. Masojidek, J.; Sergejevova, M.; Soucek, P.; Kopecka, J. Equipment for Phototrophic Cultivation of Microalgae. CZ Pat. 304988, PV2013-803 (in Czech Only). 2015. Available online: https://isdv.upv.cz/doc/FullFiles/Patents/FullDocuments/304/304988.pdf (accessed on 15 January 2020).
29. Doucha, J.; Livansky, K. Bioreactor for Outdoor Thin-Layer Cultivation of Algae and Blue-Green Algae. CZ ut. Model 9966, PV2000-10401 (in Czech Only). 2000. Available online: https://isdv.upv.cz/doc/FullFiles/UtilityModels/FullDocuments/FDUM0009/uv009966.pdf (accessed on 15 January 2020).
30. Centre Algatech. Photoautotrophic Cultivation of Microalgae. ©2014a. Available online: https://www.alga.cz/en/c-89-jiri-masojidek-s-group.html/ (accessed on 12 September 2022).
31. Centre Algatech. Heterotrophic Cultivation of Algae. ©2014b. Available online: https://www.alga.cz/en/c-869-heterotrophic-cultivation-of-algae.html (accessed on 15 September 2022).
32. Doucha, J.; Livansky, K. Method of Controlled Cultivation of Algae in Heterotrophic Nutrition Mode. CZ Pat. 288638, PV1998-1007 (in Czech Only). 2001. Available online: https://isdv.upv.cz/doc/FullFiles/Patents/FullDocuments/288/288638.pdf (accessed on 12 January 2020).

33. Ruiz, J.; Wijffels, R.H.; Dominguez, M.; Barbosa, M.J. Heterotrophic vs autotrophic production of microalgae: Bringing some light into the everlasting cost controversy. *Algal Res.* **2022**, *64*, 102698. [CrossRef]
34. Subitech. Technology (Flat Panel Airlift Photobioreactor). ©2022b. Available online: https://www.subitec.com/en/industry/technology-fpa/ (accessed on 31 September 2022).
35. Algamo: About Us. Our Production and Technology. ©2019. Available online: https://www.algamo.cz/index.php/en/homepageen/ (accessed on 31 September 2022).
36. Subitech. Production Plant. ©2017. Available online: https://subitec.com/en/production-plants-large-scale-algae-cultivation (accessed on 7 November 2020).
37. Hata, N.; Ogbonna, J.C.; Hasegawa, Y.; Taroda, H.; Tanaka, H. Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic-photoautotrophic culture. *J. Appl. Phycol.* **2001**, *13*, 395–402. [CrossRef]
38. Park, J.C.; Choi, S.P.; Hong, M.E.; Sim, S.J. Enhanced astaxanthin production from microalga, *Haematococcus pluvialis* by two-stage perfusion culture with stepwise light irradiation. *Bioprocess Biosyst. Eng.* **2014**, *37*, 2039–2047. [CrossRef]
39. *ISO 14040:2006-ed.2.0/Amd1:2020*; Environmental Management—Life Cycle Assessment—Principles and Framework. ISO/TC2 07/SC 5; ISO: Geneva, Switzerland, 2020; Volume 22.
40. *ISO 14044:2006/Amd1:2017/Amd2:2020*; Environmental Management—Life Cycle Assessment—Requirements and Guidelines. ISO/TC2 07/SC 5; ISO: Geneva, Switzerland, 2020; Volume 56.
41. Finnveden, G.; Hauschild, M.Z.; Ekvall, T.; Guinée, J.; Heijungs, R.; Hellweg, S.; Koehler, A.; Pennington, D.; Suh, S. Recent developments in life cycle assessment. *J. Environ. Manag.* **2009**, *91*, 1–21. [CrossRef]
42. Sills, D.L.; Van Doren, L.G.; Beal, C.; Raynor, E. The effect of functional unit and co-product handling methods on life cycle assessment of an algal biorefinery. *Algal Res.* **2020**, *46*, 101770. [CrossRef]
43. Guinée, J.B.; Heijungs, R.; Huppes, G.; Zamagni, A.; Masoni, P.; Buonamici, R.; Ekval, T.; Rydberg, T. Life cycle assessment: Past, present, and future. *Environ. Sci. Technol.* **2011**, *45*, 90–96. [CrossRef]
44. Guinée, J.B.; Gorrée, M.; Heijungs, R.; Huppes, G.; Kleijn, R.; de Koning, A.; van Oers, L.; Wegener Sleeswijk, A.; Suh, S.; Udo de Haes, H.A.; et al. *Handbook on Life Cycle Assessment*; Operational Guide to the ISO Standards; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2002; ISBN 1-4020-0228-9.
45. Acero, A.P.; Rodriguez, C.; Ciroth, A. *LCIA Methods—Impact Assessment Methods in Life Cycle Assessment and Their Impact Categories*; Version 1.5.6; GreenDelta: Berlin, Germany, 2017; Available online: https://www.openlca.org/wp-content/uploads/2015/11/openLCA_LCIA_METHODS-v.1.5.6.pdf (accessed on 20 October 2021).
46. Dreyer, L.C.; Niemann, A.L.; Hauschild, M.Z. Comparison of three different LCIA methods: EDIP97, CML2001 and Eco-indicator 99. Does it matter which one you choose? *Int. J. Life Cycle Assess.* **2003**, *8*, 191–200. [CrossRef]
47. Vavrova, K.; Wimmerova, L.; Knapek, J.; Weger, J.; Keken, Z.; Kastanek, F.; Solcova, O. Waste feathers processing to liquid fertilizers for sustainable agriculture—LCA, economic evaluation, and case study. *Processes* **2022**, *10*, 2478. [CrossRef]