




Review

Microalgae Cultivation Technologies as an Opportunity for Bioenergetic System Development—Advantages and Limitations

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Abstract: Microalgal biomass is currently considered as a sustainable and renewable feedstock for biofuel production (biohydrogen, biomethane, biodiesel) characterized by lower emissions of hazardous air pollutants than fossil fuels. Photobioreactors for microalgae growth can be exploited using many industrial and domestic wastes. It allows locating the commercial microalgal systems in areas that cannot be employed for agricultural purposes, i.e., near heating or wastewater treatment plants and other industrial facilities producing carbon dioxide and organic and nutrient compounds. Despite their high potential, the large-scale algal biomass production technologies are not popular because the systems for biomass production, separation, drainage, and conversion into energy carriers are difficult to explicitly assess and balance, considering the ecological and economical concerns. Most of the studies presented in the literature have been carried out on a small, laboratory scale. This significantly limits the possibility of obtaining reliable data for a comprehensive assessment of the efficiency of such solutions. Therefore, there is a need to verify the results in pilot-scale and the full technical-scale studies. This study summarizes the strengths and weaknesses of microalgal biomass production technologies for bioenergetic applications.

Keywords: microalgal biomass; microalgae cultivation; biofuels; advantages; limitations

1. Introduction

Microalgae are single-cell organisms that convert solar radiation energy into chemical energy via photosynthesis [1]. Controlled production of microalgal biomass is a fast-growing technology, as microalgae can be used to produce a wide range of commercially valuable cellular metabolites, including high-quality proteins, lipids, carbohydrates, dyes, and vitamins for the food/feed industry and the broad cosmetic industry (Table 1).

The fact that microalgae represent an alternative and competitive source of biomass is due to their advantage over typical terrestrial and energy plants [2]. Algae possess very high photosynthetic efficiency [3], can relatively fast build biomass [4], are resistant to various contaminants [5], and can be grown on land that is unsuitable for other purposes [6]. Microalgae production systems can also be used in environment-protecting technologies [7], including sewage and leachate treatment [8], neutralization of waste and sludge [9], carbon dioxide biosequestration, biogas upgrading, and flue gas treatment [10] (Table 2). This makes it possible to select and adapt specific strains for individual

applications, including energy carrier production, environmental protection, and environmental engineering technologies [11]. Given these considerations, algae may provide a viable alternative to traditional energy crops [12].

Table 1. Venues for microalgal biomass application.

Product		Use	Ref.
Agar		Food ingredient, fruit preserves, hydrocolloids, clarifying brewing agent, paper industry, and others	[13]
Alginate		Food additive, medical, pharmaceutical, paper, cosmetic and fertilizer industries, textile printing	[1]
Antioxidants		Preservatives in cosmetic, chemical, food, and pharmaceutical industries	[14]
Astaxanthin		Food supplement as food dye additive and antioxidant	[15]
Beta-carotene and carotenoids		Precursor for vitamin A and supplement for vitamin C, food additive as coloring agent, and antioxidant	[16]
Bioenergy and biofuels		Biodiesel, bioethanol, biogas, biohydrogen, biomethane, aviation gas, biobutanol, biosyngas, bio-oil, gasoline, solid fuel, jet fuel	[15]
Biochar		Agricultural and sorbent uses, combustion	[13]
Biorefinery		Various chemicals and biofuels	[14]
Biosorbents		Ion exchange materials that bind strongly heavy metal ions	[1]
Carragen or carrageenan		Pet food, food additive, gels, toothpaste	[1]
Catalysts		Catalytic properties	[14]
Chemicals		Industrial and medicinal uses	[1]
Conditioners		Chemical, cosmetic, and farming industries	[15]
Digester residue		Compost or vermicompost	[13]
Extraction of	hydrocolloids or gums	Food industry, phytocolloids such as agar, alginate, and carrageenan	[14]
	lipids carbohydrates starch and cellulose	Biogas, biodiesel, gasoline, jet fuel, alcohols, renewable hydrocarbons	
	minerals and trace elements	Food supplements, glass production, metallurgy	
	proteins	Fertilizers, industrial enzymes, animal/fish feeds, surfactants, bioplastics	
Feed		Animal food	[15]
Fertilizers		N-, P-, and K-rich fertilizers	[1]
Phytosterol		Food supplements	[15]
Pigments		Natural colorants in paper and textile industries	[14]
Production	Cosmetic	Water-binding agents and antioxidants, “skin foods”	[15]
	Food and drink	Nori, kombu, wakame, cheese, soup, noodles, pasta, wine, tea, others	
	Fruit and vegetable preservatives	Food industry	
	Glass	Glass industry	
	Paper pulp supplements	Paper industry	
	Textile	Textile industry	
	Therapeutic materials	Pharmaceutical industry	

Table 2. Applications of microalgae in environment-protecting technologies.

Sector	Use		Ref.
Wastewater treatment	Nitrogen and phosphorus removal from municipal wastewater		[17]
	Biodegradation of sparingly degradable pollutants		[18,19]
	Treatment of organic wastewater		[20]
	Treatment of hard-to-manage wastewater produced by	timber and paper industry	[21,22]
		textile industry	[23]
		phenol industry	[24,25]
	Ethanol and citric acid production		[26]
Removal of heavy metals (copper, nickel, lead) from wastewater		[19,27]	
Gas treatment	Reducing emissions of carbon dioxide and other pollutants (nitrogen and sulfur oxides) from waste and exhaust gases		[28,29]
Waste management	Use of waste glycerol as a carbon source in heterotrophic cultivation		[30]
	Microalgae cultivation using industrial waste or low-value feedstocks, such as	breadcrumbs	[31]
		brewer's spent yeast	[32]
		coconut water	[33]
		empty palm fruit bunches	[34]
Leachate treatment	Biodegradation of landfill leachates		[35]
	Neutralization of degraded effluent from anaerobic fermentation of sewage sludge		[36]
Biogas upgrading	Biological sequestration of CO ₂ with photosynthetic microalgae (photosynthesis allows producing biogas with 94% methane content)		[37]

However, the most promising frontiers for microalgae concern their utility for energy purposes, including the production of biogas, biohydrogen, bioethanol, and biodiesel [38]. Microalgal biomass is undoubtedly a promising substrate for energy carrier production, characterized by lower pollutant emission levels compared to conventional fuels [39,40]. By way of example, forecasts for the United States (US) biofuel and biodiesel market [41,42] are shown in Figure 1.

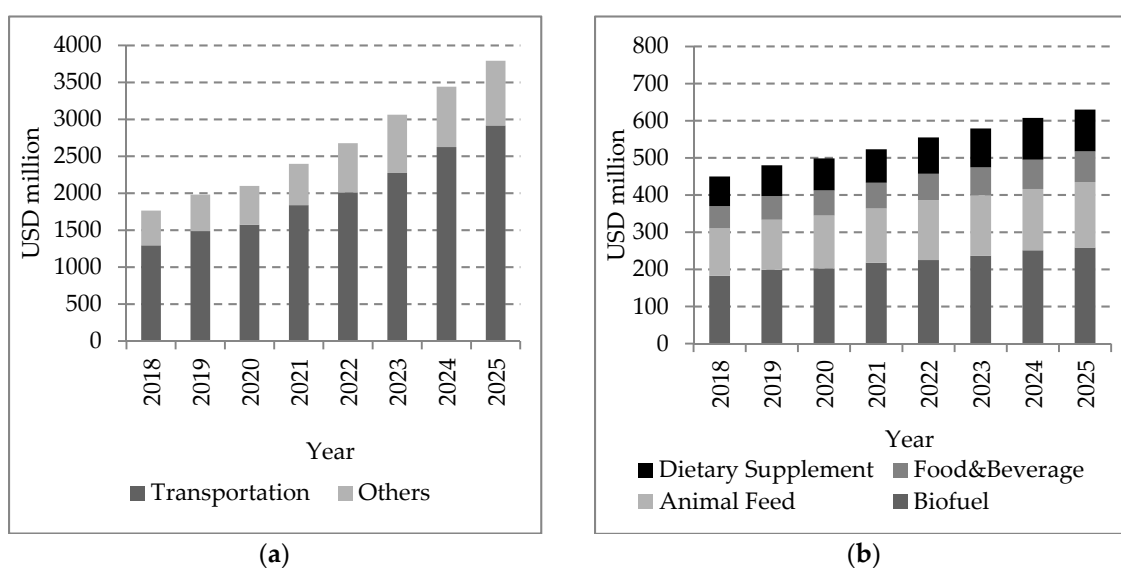


Figure 1. Growth forecasts for the United States of America (USA) algae-based biofuel market by 2025 ((a)—algae biofuel market, (b)—algae oil market).

Systems for producing algal biomass feature high technological efficiency, owing to the significant photosynthesis efficiency of algae and the relatively fast growth of algal biomass [43]. Phototrophic cultivation of microalgae in photobioreactors can process waste from industrial and municipal sources, which means that commercial microalgae cultivation systems can be constructed on land unsuitable for agricultural use, near heating/cogeneration plants, sewage treatment plants, and other industrial facilities that produce carbon dioxide and biogenic compounds [44].

Despite the demonstrated utility of microalgal biomass-based systems for the bioenergy industry, most industrial microalgae cultivation plants established and extensively described in the literature deal mainly with the production of high-quality feed/food additives, precious dyes, or fertilizing substances (Table 1), due to the difficulties in conclusively assessing and balancing methods for microalgal biomass production and technologies for converting it to energy carriers [45]. The majority of studies were carried out in laboratory conditions, with semi-industrial (pilot-scale) projects being a rarity [46]. The few examples of small-scale bioreactors are presented in Table 3 and pilot installations in Table 4.

The commercialization of technology and its transfer from laboratory conditions to a technical scale requires extensive research, conceptual, operational, and marketing works that allow the product to be finally placed on the market. Although relative studies present various models of knowledge and technology commercialization, they also show some similarities, as they involve a certain repetitive group of activities [47]. An important element in the process of making investment decisions regarding the commercialization of innovative products is to assess the maturity of new technologies. This assessment, called “technology readiness assessment” (TRA), should take into account the state of work on the development of a new product/technology, prospects for further development, the amount of funds necessary to invest, and innovative risk. It is a universal metric used to analyze the state of work on technologies and their readiness for commercial implementation. In turn, the “technology readiness level” (TRL) methodology sets nine levels of technology readiness and allows assessing the progress of works on new technologies [48]. It was first used in research and development (R&D) projects carried out by the National Aeronautics and Space Administration (NASA) and the US defense industry. According to TRL, technology maturity is described from the conceptualization phase of a specific solution (TRL 1) to the maturity stage (TRL 9), when this concept (as a result of research and development works) takes the form of a technological solution that can be implemented in practice, e.g., by launching production and marketing [49].

Table 3. Comparison of the efficiency of lab-scale microalgae cultivation systems; n.a., not applicable.

Microalgal Strains/Biomass	Cultivation System and Operation Mode	Cultivation Time (days)	Algal Growth, in $\text{g}\cdot\text{dm}^{-3}$ (Dry Basis) or $\text{cells}\cdot\text{cm}^{-3}$	Biomass Productivity ($\text{mg}\cdot\text{dm}^{-3}\cdot\text{day}^{-1}$)	Ref.
<i>Chlamydomonas</i> sp. SW13aLS	250 cm^3 flask; batch	30	No growth	No growth	[50]
<i>Chlorella pyrenoidosa</i> FACHB-28	30 dm^3 membrane Define if appropriate.; batch	30	0.41–0.63 $\text{g}\cdot\text{dm}^{-3}$	60–80	[51]
<i>Chlorella pyrenoidosa</i> NCIM 2738	3 dm^3 tubular PBR; batch	18	2.9 $\text{g}\cdot\text{dm}^{-3}$	260	[52]
<i>Chlorella</i> sp. (isolated from a clean lagoon)	500 cm^3 flasks; batch	28	3×10^7 $\text{cells}\cdot\text{cm}^{-3}$	n.a.	[53]
<i>Chlorella</i> sp. (isolated from leachate)	350 cm^3 flasks; batch	14	0.09–0.43 $\text{g}\cdot\text{dm}^{-3}$	18–66	[54]

Table 3. Cont.

Microalgal Strains/Biomass	Cultivation System and Operation Mode	Cultivation Time (days)	Algal Growth, in g·dm ⁻³ (Dry Basis) or cells·cm ⁻³	Biomass Productivity (mg·dm ⁻³ ·day ⁻¹)	Ref.
<i>Chlorella</i> sp. (marine)	24 dm ³ tubular PBR; batch	3	2.2–2.6 × 10 ⁶ cells·cm ⁻³	n.a.	[55]
<i>Chlorella vulgaris</i> CCAP 211/11B	1 dm ³ flasks; batch	10	0.81–1.71 g·dm ⁻³	20–110	[56]
<i>Chlorella vulgaris</i> CCAP 211	2 dm ³ vertical PBR; batch	28	2.10 g·dm ⁻³	63.8	[57]
<i>Chlorella vulgaris</i> FACHB-31	Membrane PBR with two 2 dm ³ chambers; batch	8	0.95 g·dm ⁻³	240	[58]
<i>Chlorella vulgaris</i> FACHB-31	2 dm ³ tubular PBR; batch	8	0.66 g·dm ⁻³	150	[58]
<i>Chlorella vulgaris</i> FACHB-31	3 dm ³ membrane PBRs; batch	12	2.13 g·dm ⁻³	n.a.	[58]
<i>Chlorella vulgaris</i> FACHB-31	3 dm ³ tubular PBR; batch	2	No growth	No growth	[58]
<i>Chlorella vulgaris</i> and <i>Chlamydomonas reinhardtii</i>	500 cm ³ bottles; batch	28–60	0.46–1.5 g·dm ⁻³	n.a.	[59]
<i>Chlorella vulgaris</i> and <i>Chlamydomonas reinhardtii</i>	200 dm ³ open raceway pond; 5 runs; batch	32–54	0.68–1.03 g·dm ⁻³	160–440	[59]
<i>Nannochloropsis gaditana</i> , <i>Pavlova lutheri</i> , <i>Tetraselmis chuii</i> , and <i>Chetoceros muelleri</i>	2.5 and 12.5 dm ³ cylindrical PBR; batch	10	Up to 9 × 10 ⁶ cells·cm ⁻³	n.a.	[60]
<i>Oscillatoria</i> sp. (isolated from leachate)	350 cm ³ flasks; batch	14	0.43–0.81 g·dm ⁻³	44–107	[54]
<i>Picochlorum oculatum</i> UTEX LB 1998	150 dm ³ horizontal bioreactor; fed-batch; 3 cycles	18–37	1.5–1.9 g·dm ⁻³ (1.2–1.7 × 10 ⁹ cells·cm ⁻³)	37–55	[61]
<i>Scenedesmus</i> sp. (isolated from leachate)	350 cm ³ flasks; batch	14	0.16–0.24 g·dm ⁻³	37–46	[54]
<i>Scenedesmus</i> sp. CHX1	250 cm ³ flasks; batch	20	0.22 g·dm ⁻³	37.5	[62]

As such, there are very few sources of reliable data for a comprehensive evaluation of the technological, environmental, and economic efficiency of these solutions [63]. Such assessments are further complicated because various researchers have presented contradictory conclusions on microalgal biomass productivity, as well as its actual technological performance and cost-effectiveness. Lardon et al. (2009) unfavorably compared microalgal cultivation with traditional production methods, concluding that it is not a financially viable means of biodiesel production due to very high costs of biomass cultivation, harvesting, and drying, as well as of oil extraction [64]. On the other hand, Clarens et al. (2011) demonstrated the opposite, obtaining a positive energy balance and a beneficial environmental outcome for the biodiesel produced from microalgal biomass. They used exhaust gases and wastewater as sources of carbon dioxide and biogenic compounds for the growth medium [65]. In turn, Frank et al. (2011) used computational software to create a model that demonstrated microalgal

fuel production technologies to be less energy-efficient and producing more greenhouse gases than traditional biofuel production methods [66].

Table 4. Pilot projects concerning microalgal biomass production and its conversion to energy carriers. PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid.

Projects/Research Institutes	Focal Area	Ref.
Algae Innovation Center, Green Centre, Denmark	Demonstration and test projects concerning algae cultivation; an assessment of biomass viability was conducted on the site (in Rødsand II)	[67]
Algenol, USA	Production of bioethanol and pigments in a raceway pond, and closed and semi-closed bioreactors	[68]
Algatechnologies, Israel	Production of astaxanthin in closed and semi-closed bioreactors under high light intensity	[69]
Algenol Biotech, LLC	An integrated, pilot project involving the photosynthetic production of ethanol and the delivery of a photobioreactor system that can be scaled for commercial operation	[70]
BioReal Inc, USA	Production of astaxanthin in an indoor photobioreactor	[71]
BioProcess Algae, LLC	A pilot project on growing low-cost algae using renewable CO ₂ , lignocellulosic sugars, and waste heat provided by a co-located ethanol plant	[70]
Blue Bio Projekt (IVA Kattegat-Skagerrak)	Finding sustainable ways of exploiting microalgae	[67]
Cellana, USA	Production of PUFAs, animal feed, biodiesel, and bio jet fuel in an open-pond bioreactor	[72]
Cyanotech, Hawaii	Production of astaxanthin from <i>Spirulina pacifica</i> as a food ingredient in a raceway pond and photobioreactors	[69]
IGV GmbH, Germany	Algae cultivation in a photobioreactor	[67]
Kingfisher, Sweden	Tested equipment (including offshore wind parks) for offshore mussel and algae cultivation	[67]
Mera Pharmaceuticals Incorporation	Production of astaxanthin from <i>Haematococcus pluvialis</i> in a raceway pond	[73]
Muradel Pty Ltd., Australia	Production of biofuels, oleochemicals, biofertilizers, animal feed, and building materials in a raceway pond	[74]
Sea6 Energy, India	Production of food additives, biofuel, bioplastic, and animal feed in sea water	[75]
Sapphire Energy Inc. USA	A demonstration-scale project involving the construction and operation of a 100-acre algae farm and conversion facility for the production of renewable bio-crude	[70]
Solazyme Inc. USA	An integrated pilot project involving heterotrophic algae that can convert cellulosic sugars to diesel fuel	[70]
Solix Algadrients Inc., USA	Production of astaxanthin and DHA in enclosed photobioreactors	[76]
RWE Power AG, Germany	Flue gas used to grow algae in a demonstration project	[67]
Technical Research Centre of Finland	Design and validation of a new integrated “biowaste-to-energy” concept involving algae cultivation and biogas production	[67]
University of Warmia and Mazury in Olsztyn, Poland	Cultivation of lipid-rich microalgal biomass as anaerobic digestate valorization technology—a pilot-scale study	[77]

It bears repeating that the research conducted to date was either small-scale or limited to theoretical analyses based on literature data. While these kinds of studies can yield interesting results, they do not provide enough information to properly and exhaustively analyze how such systems perform in operation. Therefore, there is a legitimate need to verify the results obtained in laboratory conditions by launching and operating pilot-scale and full-scale installations. The present paper draws on currently available data to summarize the strengths and weaknesses of biofuel production technologies based on microalgal biomass cultivation.

2. Microalgal Biomass as a Source of Biofuels

Microalgae can serve as a potential source of many different types of biofuels (Figure 2). Examples include anaerobic digestion of biomass into biogas, production of biodiesel from lipids stored in algae cells and hydrogen from photobiological conversion, and lastly, gasification, pyrolysis, or direct combustion of the harvested algal biomass [78,79].

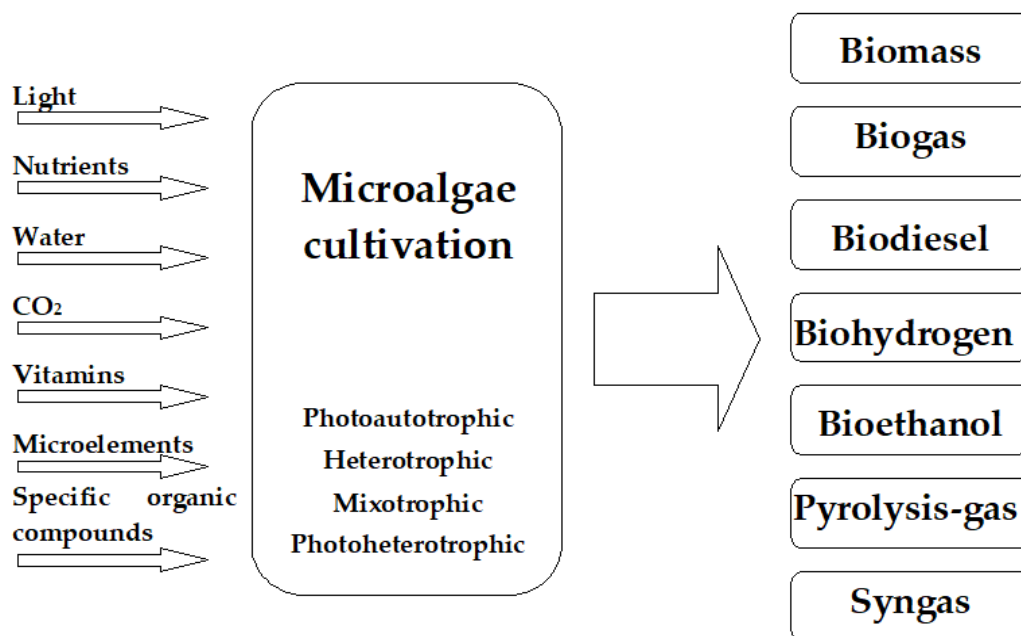


Figure 2. Available mechanisms for producing biofuel with microalgae.

The simplest way to use microalgae for fuel purposes involves the combustion or co-combustion of their pre-dried biomass [80]. However, this solution is rarely practiced, most often in cases where the biomass of microalgae cannot be used to produce more advanced biofuels [81]. Biogas and biomethane are produced during controlled, anaerobic degradation of microalgal biomass by fermentation bacteria [82]. Methane fermentation is a cascade of successive biochemical transformations, including hydrolysis, acidogenesis, and methanogenesis, which are carried out by specialized consortia of microorganisms [83]. In turn, biodiesel is produced via the transesterification of bio-oil extracted from microalgal biomass. This process involves the reaction of triglyceride molecules, bio-oil components, with low-molecular-weight alcohols in the presence of catalysts [84]. Hydrogen production by microalgae is based on direct biophotolysis, which involves the photosynthetic production of hydrogen from water, which uses the energy of light to break down the water molecule into hydrogen and oxygen. The process is mediated by hydrogenase—a metal enzyme that catalyzes the reversible oxidation of H₂ and releases gaseous hydrogen by reducing protons [85]. The basic technology for bioethanol production from microalgae entails a biochemical process in which bacteria hydrolyze the biomass and then yeast convert the sugars present in the biomass into alcohol, which is then distilled and dehydrated [86]. In turn, syngas and pyrolytic gas are produced via the endothermal conversion of biomass into gas, which mainly consists of hydrogen, carbon monoxide, carbon dioxide, methane, and low-molecular-weight hydrocarbons [87]. The contribution of individual products, including their qualitative composition, depends mainly on the process conditions, such as temperature, reaction time, pressure, and biomass characteristics [88].

The research conducted so far has shown that some microalgae strains have the ability to store substantial quantities of lipids in their cells, with lipid content accounting for even as much as 20–50% of dry matter (Table 5).

Table 5. Production of bio-oil from microalgal biomass. DM, dry matter.

Microalgal Strains/Biomass	Type of Culture	Biomass Yield (g _{DM} ·dm ⁻³ ·day ⁻¹)	Lipid Yield (mg·dm ⁻³ ·day ⁻¹)	Ref.
<i>Asteromonas gracilis</i>	Phototrophic	0.04	8.25	[89]
<i>Botryosphaerella</i> sp. AVFF007 (floating cells)	Phototrophic	0.16	46	[90]
<i>Chaetoceros muelleri</i> F&M-M43	Phototrophic	0.07	21.8	[91]
<i>Chlamydomonas</i> sp. YQJ-1	Phototrophic	0.06	20	[92]
<i>Chlamydomonas reinhardtii</i>	Phototrophic	0.05	10	[93]
<i>Chlorella emersonii</i>	Phototrophic	0.29	55	[93]
<i>Chlorella minutissima</i> UTEX 2341	Phototrophic	0.02–0.03	9.0–10.2	[94]
<i>Chlorella protothecoides</i>	Heterotrophic	4.0–4.4	1881.3–1840.0	[95]
<i>Chlorella protothecoides</i>	Heterotrophic	2	932	[96]
<i>Chlorella vulgaris</i> #259	Mixotrophic	0.09–0.25	22.0–54.0	[97]
<i>Chlorella vulgaris</i> CCAP 211/11B	Phototrophic	0.17	32.6	[91]
<i>Desmodesmus</i> sp. DZL-4	Phototrophic	0.16	50	[92]
<i>Dunaliella salina</i>	Phototrophic	0.05	10	[93]
<i>Micractinium</i> sp. IR-4	Phototrophic	0.11	20	[92]
<i>Monoraphidium</i> sp. QLY-1	Phototrophic	0.02	11.6	[98]
<i>Monoraphidium</i> sp. QLZ-3	Phototrophic	0.03	7.2	[98]
<i>Monoraphidium</i> sp. YLY-2	Phototrophic	0.01	4.9	[98]
<i>Nannochloropsis</i> sp. F&M-M29	Phototrophic	0.17	37.6	[91]
<i>Pavlova salina</i> CS 49	Phototrophic	0.16	49.4	[91]
<i>Phaeodactylum tricornutum</i> F&M-M40	Phototrophic	0.24	44.8	[91]
<i>Scenedesmus obliquus</i>	Mixotrophic	0.10–0.51	11.6–58.6	[99]
<i>Scenedesmus obliquus</i>	Phototrophic	0.06	7.14	[99]
<i>Scenedesmus quadricauda</i>	Phototrophic	0.19	35.1	[91]
<i>Scenedesmus</i> sp. DM	Phototrophic	0.26	53.9	[91]
<i>Scenedesmus</i> sp. F&M-M19	Phototrophic	0.21	40.8	[91]
<i>Schizochytrium</i> sp. S31	Phototrophic	0.88	100.7	[100]
<i>Selenastrum</i> sp. XL-3-3		0.22	130	[92]
<i>Skeletonema costatum</i> CS 181	Phototrophic	0.08	17.4	[91]
<i>Tetraselmis suecica</i> F&M-M33	Phototrophic	0.32	27	[91]
<i>Thalassiosira pseudonana</i> CS 173	Phototrophic	0.08	17.4	[91]
<i>Thalassiosira</i> sp.	Phototrophic	0.02–0.03	10.4	[101]
<i>Thraustochytrium</i> sp. BM2	Heterotrophic	2.13	1683	[102]
<i>Thraustochytrium</i> sp. CR01	Heterotrophic	2	1140	[103]
<i>Thraustochytrium striatum</i>	Heterotrophic	0.4	52	[104]

There are also reports describing technologies that stimulate and increase fatty compound storage through controlling the concentration of nitrogen compounds in the growth medium, adjusting the supply of light energy, regulating the temperature conditions, and changing the CO₂ levels [105,106]. Essential prerequisites for cost-effective biodiesel production include the development of economically feasible technologies for separation/thickening of algal biomass, as well as oil extraction methods [107]. The temperature of the extraction process is a crucial factor that directly affects the quality and quantity of the resultant oil [108]. At temperatures of 60 °C and lower, higher triglyceride levels are achieved, and oil losses are reduced. Although the common practice of lipid extraction is mainly based on the use of organic solvents, some alternative and competitive technologies are still being sought. Other independent methods that aid the extraction process include mechanical, chemical, and biological treatments [109]. Despite being simple, environmentally friendly, and cheap, the mechanical methods offer a low lipid recovery efficiency [110]. Thus, intensive research works are in progress on the use of ionic liquids, supercritical fluids, bio-based extractants, and switchable solvents with simultaneous attention paid to reducing the energy consumption of the process by eliminating the energy-intensive drying process and the integration of multiple downstream processing steps [111]. A prospective solution for lipid recovery is offered by hybrid methods, e.g., enzymatic and mechanical/solvent

extraction [112]. The selection of a suitable method for efficient lipid extraction largely depends on the biology and cell-wall characteristics of microalgae [113].

Technologies for converting algal biomass into energy carriers can be divided into two main groups related to thermochemical and biochemical processing [114,115]. Gasification is one of the thermochemical routes, wherein biomass is partially oxidized at temperatures ranging from 800 to 1000 °C [116]. This technological solution entails reacting the biomass with oxygen and water vapor, which directly results in the generation of syngas—a mixture of CO, H₂, CO₂, N, and CH₄ [117]. Syngas has a low calorific value, ranging from 4.0 to 6.0 MJ·m⁻³, and can be combusted directly or used as a fuel in gas turbines and gas engines [118]. The properties and parameters of the microalgal biomass gasification process have been identified by several researchers. A study by Hirano et al. (1998) examined the gasification of *Spirulina* sp. algae at temperatures between 850 and 1000 °C and compared the obtained energy value of syngas with that of methanol. The highest operational performance was achieved with a gasification temperature of 1000 °C [119]. Minowa and Sawayama (1999) gasified *Chlorella vulgaris* algae within a novel technological system, producing high-methane biofuel, as well as a fertilizer rich in ammonium nitrogen [120].

A different technology for obtaining liquid biofuel is based on thermochemical liquefaction of algal biomass [121]. The process is conducted at 300–350 °C and 5.0–20.0 MPa. Thermochemical reactions are induced in the presence of hydrogen, which serves as a catalyst [122]. The reactors are complex, both design- and technology-wise, which directly affects the construction and operation costs [123]. Dote et al. (1994) successfully used the featured technology to process *Botryococcus braunii* algae and obtained an oil yield of 64.0% dry matter of the algae fed into the reactor. The heating value of the bio-oil was 45.9 MJ·kg⁻¹, with a positive energy balance achieved across the entire process [124]. In a similar experiment with *Dunaliella tertiolecta*, a bio-oil recovery yield reached 42.0% dry algal biomass, and the calorific value of the resulting product was 34.9 MJ·kg⁻¹ [125].

Pyrolysis is yet another technology used to convert algal biomass into biofuel (Table 6). Compared with the other methods presented in the literature, it has been widely described as a promising technology that yields very good results, inspiring high hopes for application in full-scale installations [126]. Miao and Wu (2004a) used pyrolysis to extract oil from heterotrophic cultures of *Chlorella prothothecoides* microalgae and achieved a bio-oil yield of 57.9% algal dry matter, with the calorific value of the resultant biofuel averaging 41.0 MJ·kg⁻¹ [127]. By comparison, Miao et al. (2004b) produced bio-oil having a calorific value of 30.0 MJ·kg⁻¹ at a yield of 18.0% dry *Chlorella prothothecoides* biomass and 29.0 MJ·kg⁻¹ at a yield of 24.0% dry *Microcystis aeruginosa* biomass. The algae were grown in autotrophic conditions [128].

Table 6. Studies on slow pyrolysis of microalgae.

Microalgal Strains/Biomass	Oil Recovery		Ref.
	Temperature (°C)	Efficiency (%)	
<i>Chlorella</i>	425	35.0	[129]
<i>Chlorella vulgaris</i>	500	49.2	[130]
<i>Chlorella vulgaris</i>	500	41.0	[131]
<i>Chlorella protothecoides</i>	500	55.3	[132]
<i>Chrysophyceae</i>	450	49.4	[129]
<i>Cladophora</i> sp.	600	20.0	[133]
<i>Dunaliella salina</i>	500	55.4	[130]
<i>Lyngbya</i> sp.	600	13.0	[133]
<i>Microcystis</i> sp.	500	54.97	[134]
<i>Spirulina</i>	425	40.6	[129]
<i>Spirulina platensis</i>	350–500	23.0–29.0	[135]

Demirbas (2006) experimented with the pyrolysis of *Chlorella prothotecooides* algae, aiming to ascertain how the efficiency of the process changed with temperature. The efficiency of oil recovery from pyrolyzed algal dry matter increased from 5.7% to 55.3% as the temperature rose from 254 to 502 °C. Further increases in temperature led to a direct reduction in production yields. The heating value of the harvested bio-oil peaked at 39.7 MJ·kg⁻¹ [136]. Many of the findings published in the literature seem to indicate that bio-oil extracted from algal biomass is higher in quality than the biofuel obtained through pyrolysis of lignocellulosic plants [136,137].

Algae can also serve as a source of ethyl alcohol (Table 7). It has been demonstrated that *Chlorella* sp. algae are viable candidates for effective alcoholic fermentation due to their high starch content (approximately 37.0% dry matter). Experimental data indicate a carbohydrate-to-ethanol conversion rate of 65.0% [138]. Ueno et al. (1998) corroborated the feasibility of ethanol production using microalgae harvested from a heterotrophic culture. The productivity of the alcoholic fermentation process performed at 30 °C was 450 µmol·g⁻¹ dry matter [139]. The research carried out to date confirms that the production of ethyl alcohol from algal biomass can be technologically and commercially viable under specific conditions. In most cases, however, alcoholic fermentation is used as a supplemental technological step for processing algal biomass residues from the oil extraction process [140].

Table 7. Ethanol yields from microalgae using different fermentative microorganisms.

Microalgal Strains/Biomass	Pretreatment	Fermentative Microorganism	Fermentation Condition				Ethanol Production		Ref.	
			Process	Temperature (°C)	Time	pH	Agitation (rpm)	Max		Units
<i>Chlamydomonas reinhardtii</i> UTEX 90	Enzymatic	<i>Saccharomyces cerevisiae</i> S288C	SSF	30	40 (h)	-	160	0.235	(g·g ⁻¹ algae)	[141]
<i>Chlorella</i>	Chemical (HCl and MgCl ₂)	<i>Saccharomyces cerevisiae</i> Y01	-	30	48 (h)	-	200	22.60	(g·dm ⁻³)	[142]
<i>Chlorella variabilis</i>	Viral and enzymatic	<i>Escherichiacoli</i> KO11	-	35	3 (days)	6.5	150	0.326	(g·g ⁻¹ carbohydrate consumed)	[143]
<i>Chlorella vulgaris</i>	Chemical (H ₂ SO ₄)	<i>Escherichiacoli</i> SJL2526	SHF	37	-	7.0	170	0.4	(g·g ⁻¹ algae)	[144]
<i>Chlorella vulgaris</i> FSP-E	Chemical (H ₂ SO ₄)	<i>Zymomonasmobilis</i> ATCC 29191	SHF	30	12 (h)	5–6	-	11.66	(g·dm ⁻³)	[145]
<i>Chlorococcum infusionum</i>	Chemical (NaOH)	<i>Saccharomyces cerevisiae</i>	-	72	-	-	200	0.26	(g·g ⁻¹ algae)	[146]
<i>Chlorococum</i> sp.	Supercritical fluid	<i>Saccharomyces bayanus</i>	-	30	60 (h)	-	200	3.83	(g·dm ⁻³)	[147]
<i>Porphyridium cruentum</i>	Enzymatic	<i>Saccharomyces cerevisiae</i> KCTC 7906	SSF	37	9 (h)	4.8	-	2.77 2.98	(g·dm ⁻³) (seawater) (g·dm ⁻³) (freshwater)	[148]
<i>Scenedesmus obliquus</i> CNW-N	Chemical (H ₂ SO ₄)	<i>Zymomonasmobilis</i> ATCC29191	SHF	30	4 (h)	6	-	8.55	(g·dm ⁻³)	[145]

SSF—simultaneous saccharification and fermentation; SHF—separate hydrolysis and fermentation.

Hydrogen is a naturally occurring molecule that can serve as a clean and efficient energy carrier. Studies have confirmed that microalgae possess the genetic, metabolic, and enzymatic properties required to produce H_2 through biochemical conversion [149]. Under anaerobic conditions, eukaryotic algae generate hydrogen as an electron donor in their metabolic pathways as part of the CO_2 fixation process. This mechanism has been found to occur both in the light and in the absence of any light sources [150]. During photosynthesis, algae convert the water molecule into a hydrogen ion (H^+) and oxygen. The H^+ ions are then converted by hydrogenase into molecular hydrogen (H_2) under anaerobic conditions [151]. It has been demonstrated that, if photosynthesis is initiated and oxygen is present in the photosynthetic environment, inhibition of the key enzyme (hydrogenase) follows shortly, directly affecting hydrogen production by algae [152].

Most of the scientific publications on this subject reported that the single-cell *Chlamydomonas reinhardtii* algae, commonly found in soil and saltwater, can produce H_2 with high efficiency (Table 8) [153,154]. The hydrogen production capacity of 21 green algae species in an isolated anaerobic environment was also examined. The most productive strains were *C. reinhardtii*, *C. euryale*, *C. noctigama*, *C. vectensis*, *C. pyrenoidosa*, *Oocystis*, *D. subspicatus*, and *P. subcapitata*. Publications reported H_2 yields of $90\text{--}110\text{ cm}^3\text{ H}_2\cdot\text{dm}^{-3}$ for these organisms, with even higher levels of $80\text{--}140\text{ cm}^3\text{ H}_2\cdot\text{dm}^{-3}$ reached in some cases [155]. Ample publications have shown that *Platymonas subcordiformis* algae can be used for the technological production of biohydrogen (Table 8). The method employs alternating dark and light cycles with external carbon dosing, and it can produce H_2 yields of $78.0\text{ cm}^3\text{ H}_2\cdot\text{dm}^{-3}$ to as high as $126\text{ cm}^3\text{ H}_2\cdot\text{dm}^{-3}$ [156,157].

Table 8. Microalgal biohydrogen production.

Microalgal Strains/Biomass	Hydrogen Yield ($\text{cm}^3\cdot\text{dm}^{-3}$)	Ref.
<i>Chlamydomonas reinhardtii</i>	5.2	[158]
<i>Chlamydomonas reinhardtii</i>	210.9	[159]
<i>Chlamydomonas reinhardtii</i>	120.0	[155]
<i>Chlorella</i> sp.	150.0	[160]
<i>Platymonas Subcordiformis</i>	50.0	[161]
<i>Platymonas Subcordiformis</i>	157.7	[162]
<i>Tetraselmis Subcordiformis</i>	55.8	[163]

Methane fermentation can also be employed to convert algal biomass into a gaseous energy carrier through biochemical processes (Table 9). According to available estimates, the conversion of algal biomass into biogas is a highly cost-effective and commercially viable technological solution comparable to cellular lipid extraction in terms of harvested energy [164,165]. In addition to high-energy biogas, the process also produces digestate, which can be used directly as a fertilizer for terrestrial plants or reintroduced into the algal biomass route as a medium component after simple processing [166].

The practical limitations of technological processes involving methane fermentation of algae may stem from their biochemical composition. Algal biomass mostly consists of proteins and, thus, may lead to deficient C:N ratios. This problem can be greatly alleviated through the co-digestion of the algal biomass with organic substrates rich in carbon compounds. Yen and Brune (2007) achieved a substantial increase in methane production by co-digesting cellulose waste with algal biomass. The methane production rate rose to $1170 \pm 75\text{ cm}^3\cdot\text{dm}^{-3}\cdot\text{day}^{-1}$ at a 1:1 ratio of organic waste and algal biomass, as compared to $573 \pm 28\text{ cm}^3\cdot\text{dm}^{-3}\cdot\text{day}^{-1}$ achieved for mono-digestion of algae alone [167].

The high protein content of the algal biomass may lead to an increased production of free ammonia, which is toxic to the methane-fermenting microorganisms. Methanogenesis can also be inhibited by the sodium ions present in the algal biomass from saltwater-based cultivation systems. However, some studies show that anaerobic sludge microorganisms can be adapted and incorporated into the process for the efficient digestion of marine algal biomass [168,169].

Table 9. Production of bio-oil from microalgal biomass.

Microalgal Strains/Biomass	Methane Fermentation Condition		Biogas Yield (cm ³ ·g ^{−1} VS)	Methane Yield (cm ³ ·g ^{−1} VS)	Ref.
	T (°C)	Time (days)			
<i>Arthrospira platensis</i>	38	32	481	293.41	[170]
<i>Botryococcus braunii</i> (pretreated biomass)	30	45	—	521	[171]
<i>Botryococcus braunii</i>	35	34–50	—	343–370	[172]
<i>Chlamydomonas reinhardtii</i>	38	32	587	387.42	[170]
<i>Chlamydomonas</i> sp.	35	34–50	—	333	[172]
<i>Chlorella kessleri</i>	38	32	335	217.75	[170]
<i>Chlorella minutissima</i>	36	—	340	166.12	[173]
<i>Chlorella pyrenoidosa</i>	36	—	464	264.71	[173]
<i>Chlorella sorokiniana</i>	30	42	—	298	[174]
<i>Chlorella sorokiniana</i> (pretreated biomass)	30	42	—	388	[174]
<i>Chlorella sorokiniana</i>	40–41	71	248	212	[175]
<i>Chlorella vulgaris</i>	36	—	369	195.64	[173]
<i>Chroococcus</i> sp.	36	30	487	267.36	[176]
<i>Dunaliella salina</i>	38	32	505	323.2	[170]
<i>Euglena gracilis</i>	38	32	485	324.95	[170]
<i>Isochrysis</i> sp.	35	34–50	—	408	[172]
<i>Macrocystis pyrifera</i>	37	31	—	545	[177]
<i>Nannochloropsis oculata</i>	35	30	—	204	[178]
<i>Nannochloropsis salina</i> (lipid extracted biomass)	37	40	—	130	[179]
<i>Scenedesmus dimorphus</i>	35	34–50	—	397	[172]
<i>Scenedesmus obliquus</i>	38	32	287	177.94	[170]

VS—volatile solids

Many researchers have argued that methane fermentation is the most promising and effective method for producing energy from algae. Sialve et al. (2009) found that, given suitable operating conditions, methane fermentation as a primary method of algal biomass processing is more economical than systems that incorporate lipid extraction and anaerobic processing of post-extraction residues [165]. Other findings suggest that the balance of methane fermentation unit operations is the most effective in terms of both the economy of the process and the pollution levels [180]. Studies have indicated that methane fermentation may be the most practical means of converting algal biomass into energy. However, Börjesson and Berglund (2006) noted that energy inputs and environmental impact varied greatly between the different methane fermentation technologies [181]. As such, an environmental life-cycle assessment (LCA) is necessary for a complete and objective evaluation of each process [182].

To meet the current challenges related to the circular bioeconomy, it is necessary to change the approach to biorefinery processes [183]. Technological, economic, and environmental efficiency improvements can be achieved by simultaneously producing many high-value products other than biofuels [184,185]. Research and development works must, therefore, be focused on finding new, more complex, and integrated production processes. Although various strategies have been proposed for converting algal biomass into fuel and fine chemicals, none have been proven to be economically viable and energy balanced [186]. Therefore, other, valuable biological products should also be searched for. In this context, the concept of microalgae biorefineries emerged with the concept of recovering multiple products from one operating process. Considering the biorefinery complexity index (BCI) as an indicator of technical and economic risk, one of the most promising seems to be the biorefinery platform based on microalgal biomass conversion into fuels, food, dietary and feed supplements, fertilizers, and pharmaceuticals [187]. A schematic diagram of a comprehensive biorefinery approach to the processing of microalgal biomass is presented below (Figure 3).

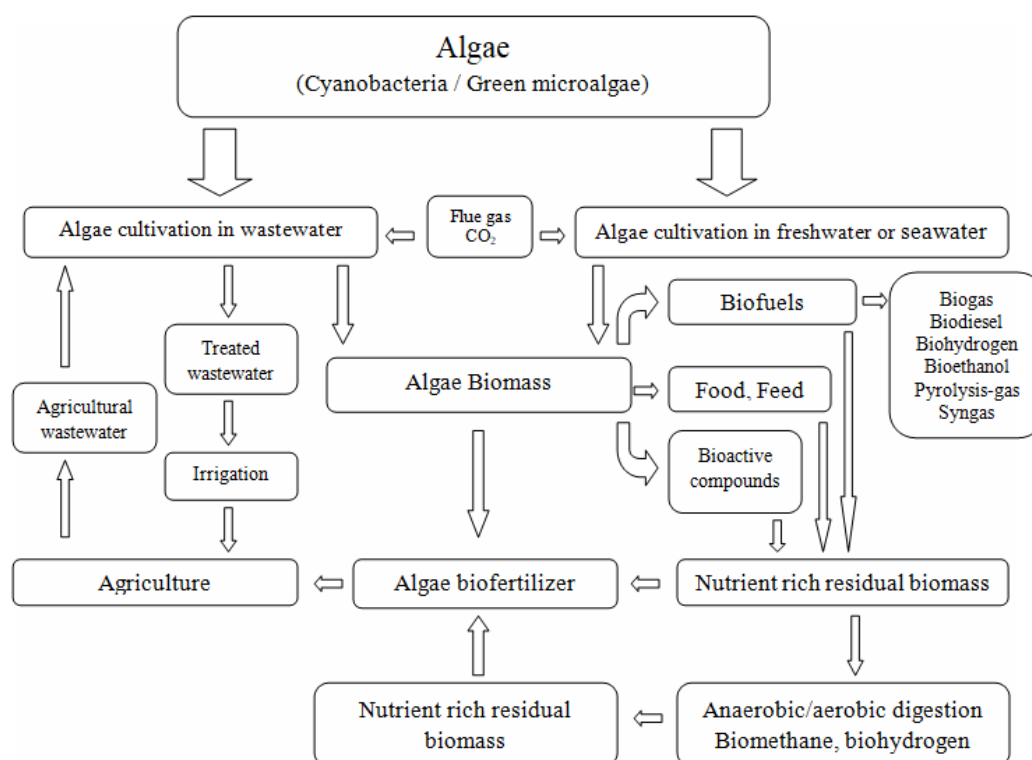


Figure 3. A schematic diagram of a comprehensive biorefinery approach to microalgal biomass processing.

3. Systems of Microalgae Species Cultivation for Biofuel

The growth rate of microalgae and their composition is influenced by the growth conditions and the species employed [188–190]. Many classification schemes categorize methods and technologies used to cultivate algae for biofuel [191,192]. Due to the specific nature of microalgae, the most important scheme divides the systems on the basis of the nutrient source and the type of biochemical processes used to grow the algal biomass rapidly. With this criterion in mind, cultures can be divided into four main types: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic [193]. The advantages and disadvantages of each type are presented in Table 10.

Table 10. Comparison of features and challenges of algal cultivation methods.

Type of Culture	Type	Advantages	Issues	Ref.
Photoautotrophic	Closed photobioreactors	Water saving Greater long-term culture maintenance High yield	High cost Temperature control (requires cooling) Maximum light exposure Periodic cleaning	[194]
	Open ponds	Lower costs Evaporative cooling	Low yield Changes in humidity and temperature Maximum light exposure	[194]
Heterotrophic	Closed photobioreactors	Easy to maintain High biomass concentrations Contamination prevention Utilization of inexpensive lignocellulosic sugars	Competition with other biofuel technologies for feedstock	[194]

Table 10. Cont.

Type of Culture	Type	Advantages	Issues	Ref.
Mixotrophic	Photobioreactor	Two-route cultivation, easy to bioremediate Uses organic compounds as energy source, provides superior energy recovery and carbon footprint	Design needs to be upgraded to improve operation and economy Rarely used for bio-oil production Not used for biodiesel production	[195–197]
Photoheterotrophic	Photobioreactor	Fast growth of algae and synthesis of valuable metabolites (i.e., fatty acids)	Requires light as an energy source, unlike mixotrophic cultivation Not used for biodiesel production	[193,196,197]

In a photoautotrophic culture, microalgae use light as their source of energy, as well as carbon dioxide and water to synthesize organic compounds [198]. This type of algae culture is most commonly used for commercial applications [199]. Studies have shown photoautotrophic cultures to exhibit great variability in algal biomass lipid content, with values ranging from 5% to 68% depending on the tested strain. A study with *Chaetoceros calcitrans* CS 178 showed a lipid production rate of $r_{LIP} = 17.6 \text{ mg} \cdot \text{dm}^{-3} \cdot \text{day}^{-1}$ and a final lipid content of 39.8% dry matter [91]. In contrast, a *Botryococcus braunii* UTEX 572 culture ended in the lipid production yield of $r_{LIP} = 5.5 \text{ mg} \cdot \text{dm}^{-3} \cdot \text{day}^{-1}$ [199]. The highest productivity was obtained in a study that tested the impact of high concentrations of CO_2 on biomass growth and lipid synthesis in *Chlorella* sp. culture. The final lipid concentration reached 32–34% cell dry matter, with a maximum lipid production rate of $r_{LIP} = 179.8 \text{ mg} \cdot \text{dm}^{-3} \cdot \text{day}^{-1}$ [200].

Like bacteria and fungi, some microalgae species are capable of heterotrophic growth using organic carbon sources, such as glucose and glycerol [196,201]. Heterotrophic cultivation can be used to avoid the problem endemic to photoautotrophic systems, i.e., overgrown photobioreactor surfaces and the microalgal growth blocking its own light source, thus limiting the energy supply necessary for efficient photosynthesis, biomass growth, and lipid synthesis [198]. Heterotrophic cultures are characterized by higher growth rates and final biomass/lipid concentrations than the phototrophic or mixotrophic cultures. For example, a heterotrophic culture of *Cryptocodinium cohnii*—a strain known for its ability to biosynthesize omega-3 acids—grown on a complex medium of glucose, acetic acid, and yeast extract, produced final concentrations of $109 \text{ g} \cdot \text{dm}^{-3}$ dry biomass and $61 \text{ g} \cdot \text{dm}^{-3}$ lipids in the culture [202].

Changing the culture conditions from photoautotrophic to heterotrophic can increase lipid content per cell dry matter for some microalgal strains. For example, a 40% increase in lipid content was observed in a *Chlorella protothecoides* culture after the cultivation scheme was changed from photoautotrophic to heterotrophic [96]. In another study, changing the conditions from phototrophic to heterotrophic led to an over tenfold reduction in the final biomass concentration in a *C. vulgaris* ESP-31 culture [203]. In the lipid analysis of *Chlorella protothecoides* cultures, Caporgno et al. (2019) achieved fatty acid contents at $11.8\% \pm 0.1\%$ dry weight (DW) and below 6% DW under heterotrophic and photoautotrophic conditions, respectively [204]. Sim et al. (2019) also observed an increased lipid production by *Chlorella protothecoides*. It reached $18.4\% \pm 0.4\%$ DW under conditions of the heterotrophic culture and $15.1\% \pm 0.3\%$ DW under photoautotrophic conditions [205]. Shen et al. (2019) demonstrated an increase in fatty acid production by *Chlorella vulgaris* that ranged from $14.9 \pm 2.1 \text{ mg} \cdot \text{dm}^{-3} \cdot \text{day}^{-1}$ under photoautotrophic conditions to $51.4 \pm 14.6 \text{ mg} \cdot \text{dm}^{-3} \cdot \text{day}^{-1}$ in the heterotrophic culture [206]. Li et al. (2016) obtained maximum biomass production in the photoautotrophic culture of *Chlorella sorokiniana*, reaching $0.36 \pm 0.01 \text{ g} \cdot \text{dm}^{-3}$ at a specific growth rate of $0.60 \pm 0.01 \text{ day}^{-1}$. Under heterotrophic conditions, the respective values were $2.78 \pm 0.06 \text{ g} \cdot \text{dm}^{-3}$ and $1.56 \pm 0.02 \text{ day}^{-1}$ [207]. In turn, Zheng et al. (2012) proved that the growth rate, cell density, and productivity of heterotrophic *Chlorella sorokiniana* were

3.0, 3.3, and 7.4 times higher than their phototrophic counterpart, respectively [208]. Lastly, Li et al. (2014) achieved the lipid content at 9.0% DW in the photoautotrophic culture of *Chlorella sorokiniana* and at 6.2% to 17.6% DW in the heterotrophic cultures [209].

Microalgae have been shown to take up many different organic carbon sources, including glucose, acetate, glycerol, fructose, sucrose, lactose, galactose, and mannose [97,210]. De Swaaf (2003) presented a study examining the use of different organic substrates in a heterotrophic culture, utilizing acetic acid and its feeding regime in a pH-controlled culture to grow *Cryptocodinium cohnii* [202]. This technological solution resulted in very high values of the final productivity parameters, i.e., final cell dry matter concentration at $109 \text{ g}\cdot\text{dm}^{-3}$ and $61 \text{ g}\cdot\text{dm}^{-3}$ lipids in the culture. Other studies showed *Chlorella protothecoides* to be capable of growth in a batch culture with crude glycerol as the sole carbon source in the medium, with the final biomass concentration at $23.5 \text{ g}\cdot\text{dm}^{-3}$ and the final lipid concentration at $14.6 \text{ g}\cdot\text{dm}^{-3}$ after a 6 day cultivation [211]. In turn, a semi-continuous batch-fed regime allowed increasing the lipid production rate to $3 \text{ g}\cdot\text{dm}^{-3}\cdot\text{day}^{-1}$ [211].

However, heterotrophic cultivation certainly has its disadvantages, including the frequent contamination of the culture with other strains of microalgae, fungi, and bacteria, reducing the final productivity of the technology and, in some cases, inhibiting fermentation [96,212,213]. One instance of this problem was described by Zhang et al. (2012) who investigated the impact of bacterial contamination on the dry biomass yield and lipid productivity in a heterotrophic culture of *Chlorella pyrenoidosa*, with soybean-processing wastewater used as a medium. On the one hand, the introduction of bacteria improved nitrogen and phosphorus degradation rates while reducing the chemical oxygen demand. On the other hand, the bacteria also reduced the final concentrations of microalgal biomass and lipids [214]. One of the methods used to avoid contamination of heterotrophic microalgal cultures entails spiking the medium with antibiotics, such as chloramphenicol [215].

In the mixotrophic cultivation, microalgal cells perform photosynthesis with simultaneous uptake of organic and inorganic carbon substrates [216]. Microalgae absorb organic compounds, and the CO_2 released through respiration is captured and reused as a substrate for photosynthesis [217]. Unlike phototrophic and heterotrophic systems, the mixotrophic cultivation is rarely employed for the production of microalgae-derived bio-oil. One example of a mixotrophic culture was found in a study by Bhatnagar et al. (2011), who examined the growth rates of *Chlamydomonas globosa*, *Chlorella minutissima*, and *Scenedesmus bijuga* in the three most common cultivation modes. Supplementing *Chlamydomonas globosa*, *Chlorella minutissima*, and *Scenedesmus bijuga* cultures with 1% (w/v) glucose was found to increase mixotrophic biomass yields 9.4, 6.7, and 5.8 times (respectively) compared to the phototrophic culture and 3.0, 2.0, and 4.4 times compared to the heterotrophic culture [218]. Yu et al. (2009) obtained similar results, demonstrating that the growth rates of *Nostoc flagelliforme* biomass in glucose-amended media were the highest in the mixotrophic culture, with productivity values 5.0 and 2.3 times those obtained in the phototrophic and heterotrophic cultures, respectively [219].

Though microalgal oil yields are in large part determined by the choice of strain, the heterotrophic cultivation is the most effective solution in terms of the final operational performance, i.e., the biomass concentration in the system and lipid content in cells. As such, the heterotrophic method has generated strong interest among companies involved in the commercialization of bioenergy technologies and research teams working to develop such systems [220]. The most serious drawback of this scheme is the risk of culture contamination with other microorganisms, including other microalgae, which leads to severe complications with the operation of industrial-scale installations [215]. Moreover, the high cost of pure organic carbon sources limits the utility of this cultivation mode to the production of secondary or primary metabolites with a high market value [221].

Photoautotrophic cultures are the most widespread mode of cultivation, easy to scale up through the use of open or hybrid systems [222]. It is also a promising method, due to the capability of photoautotrophic microalgae for the uptake of waste CO_2 , such as that generated by cogeneration plants, breweries, or biogas plants. However, the oil yields produced via this method are usually vastly inferior to the heterotrophic cultivation, with slow cell growth and low biomass productivity as

the main reasons. Nevertheless, with this mode being cheaper to scale up, it is highly attractive to investors despite the flaws.

The defining feature of photoheterotrophic cultivation is the use of light, required for the absorption and decomposition of organic carbon. The main difference between mixotrophic and photoheterotrophic modes is that the latter requires light as an energy source, whereas mixotrophic cultivation uses organic compounds for the same purpose. Therefore, photoheterotrophic cultivation requires a combined supply of carbohydrates and light [196]. Although photoheterotrophic systems can be used to increase the production of certain expensive secondary metabolites, the method has not found use in the production of biodiesel, as is the case with mixotrophic microalgal cultures [197].

Prior to undertaking any metabolic engineering work in microalgae, it is necessary to understand the key enzymes involved in the metabolic pathway and the rate-limiting enzymes. Many advances have been made toward understanding lipid metabolism and regulatory factors in soybean and rapeseed, but the lipid production in microalgae at a molecular level is currently very poorly understood. The first step in *de novo* synthesis of triacylglycerol in microalgae starts in the plastid, where pyruvate is produced from glycolysis and the Calvin cycle. The pyruvate is converted into acetyl-CoA by the pyruvate dehydrogenase complex (PDC). Acetyl-CoA is converted into malonyl-CoA by acetyl-CoA carboxylase (ACCase). Acetyl-CoA carboxylase is the rate limiting enzyme for lipid biosynthesis [223]. Malonyl-CoA is converted into malonyl-ACP by malonyl-CoA transacylase (MAT) [224]. Malonyl-ACP and acyl-ACP are converted into 3-ketoacyl-ACP by 3-ketoacyl-ACP reductase (KAS) in the fatty acid synthesis cycle. 3-Ketoacyl-ACP is converted into 3-hydroxyacyl-ACP by 3-ketoacyl-ACP reductase (KAR). 3-Hydroxyacyl-ACP is converted into *trans*-enoyl-ACP by 3-hydroxyacyl-ACP dehydratase (HD). *trans*-Enoyl-ACP is converted into acyl-ACP by enoyl-ACP reductase (ENR). Acyl-ACP is converted into free fatty acids (FFAs) by fatty acyl-ACP thioesterase (FAT) [225,226]. The FFAs are transferred into the cytosol and then endoplasmic reticulum for conversion into triacylglycerol (TAG) in the microalgae. The free fatty acids are converted into acyl-CoA by long-chain acyl-CoA synthetase. Acyl-CoA and glycerol 3-phosphate are converted into lysophosphatidic acid by glycerol 3-phosphate acyltransferase (GPAT). Lysophosphatidic acid is converted to phosphatidic acid by lysophosphatidic acid acyltransferase (LPAT). Phosphatidic acid is converted into diacylglycerol by phosphatidic acid phosphatase (PAP). Diacylglycerol is converted into triacylglycerol (TAG) by diacylglycerol acyltransferase (DGAT). Triacylglycerol forms the TAG lipid body [226,227].

Hydrogen production in biological processes conducted by algae is based on the direct biophotolysis, which consists of the photosynthetic production of hydrogen from water, in which the energy of light is used to break the water molecule into hydrogen and oxygen [228]. It takes place mainly due to hydrogenase, which catalyzes the reversible oxidation of H₂ and releases gaseous hydrogen by reducing protons [229,230]. Two transmembrane peptide complexes are responsible for hydrogen production in the photolysis process by microalgae: photosystem I (PSI) and photosystem II (PSII). The exposure of both complexes to solar radiation results in a water molecule breakdown. Then, O₂ is produced by PSII, while PSI uses the electrons generated in this process to reduce CO₂ and build cellular material (aerobic conditions), or the electrons are transferred by ferredoxin to hydrogenase and used for hydrogen production [231,232]. Another biochemical process led by algae to produce hydrogen is indirect biophotolysis. It has been proven to occur in the organisms of cyanobacteria, which accumulate carbohydrates resulting from CO₂ reduction as a result of photosynthesis, which in turn are decomposed by fermentation mediated by photosystem I. The PSI proteins transfer electrons to ferredoxin using light energy [228,232]. In the indirect biophotolysis process, an important role is played by carbon dioxide, which is a carrier of electrons and protons formed during the water molecule degradation, and by enzymes, including two NiFe hydrogenases and nitrogenase, which catalyze atmospheric nitrogen reduction to ammonia with simultaneous proton reduction and hydrogen release [233,234].

4. Strengths and Weaknesses of Different Technologies for Producing and Utilizing Microalgal Biomass

Microalgae-based technologies of sewage treatment, pollutant degradation, and biofuel production were described in detail in scientific papers, patent claims, and performance data from existing installations [235–237]. Microalgal biomass has been demonstrated to be one of the most efficient and environmentally friendly alternative energy sources, as it is a promising and sustainable source of bio-oil, methane, and biohydrogen, i.e., fuels that can help reduce atmospheric greenhouse gas emissions [238,239]. Microalgae represent an alternative to terrestrial vascular plant species commonly used as a biofuel feedstock, such as rapeseed, soybean, and oil palm [240]. Literature data indicate that the annual hectare yield of bio-oil from microalgal cultures can exceed 19 m³. By comparison, the corresponding values are 6.1 m³ for oil palm, 4.3 m³ for sugar cane, 2.4 m³ for corn, and 0.5 m³ for soybean [91,241].

The undeniable strength of the microalgae-based technologies is their well-established high photosynthetic efficiency. The efficiency of the solar-to-chemical energy conversion via algal photosynthesis varies from 4% to 10%, whereas the range for higher plants is 0.5–2.2% [235,242]. This directly translates to a fast growth rate of microalgae and a high per unit dry matter yield, significantly higher than that of terrestrial plants [235,240]. Those observations were corroborated by Tredici et al. (2015), who tested the strain *Tetraselmis suecica* in a proprietary photobioreactor design named “Green Wall Panel-II”. The research was conducted in Italy (Tuscany), with the final productivity of the culture reaching 36 tons of dry microalgal biomass·ha^{−1}·year^{−1}. By contrast, soybean grain yields are only 2.6 tons·ha^{−1}·year^{−1} [243].

Some strains of microalgae can double their mass in just a few hours. This property was described by Maxwell et al. (1994), who tested the growth rate of *Chlorella vulgaris*. The generation time observed for the species was 8.6 h at 27 °C, although cell division extended to 48.5 h at 5 °C [244]. Raslavicius et al. (2014) and Chen et al. (2015) showed that the annual microalgal biomass yields per hectare can range from 4 tons of dry matter to as high as 100 tons of dry matter [235,245]. According to other works, microalgae can double in volume or mass within a few hours, given the right conditions [238,240,242]. The resultant microalgal biomass yields can reach 500 kg·day^{−1} in a 1000 m² open pond production system [246].

One indisputable advantage of the microalgae-based solutions is that waste substrates of various properties and characteristics can be used to support rapid biomass growth [34]. Such technologies are most often used for tertiary treatment of urban or industrial waste in maturation or facultative ponds [247]. Such organisms release 1.50–1.92 kg O₂·kg^{−1} of the produced biomass through photosynthesis, with the oxygenation rate reached during degradation of organic pollutants ranging from 0.48 to 1.85 kg O₂·m^{−3}·day^{−1} [248,249]. Microalgae absorb a significant portion of the biogenic substances contained in wastewater, as they require large quantities of nitrogen and phosphorus for internal protein synthesis. As such, protein content in the algae dry matter ranges from 20% to 60%, depending on the species. The absorbed biogenic compounds are also used to synthesize nucleic acids and phospholipids [250].

Currently, microalgae-based wastewater treatment processes are often integrated into systems designed to grow algal biomass for biofuel and energy production [251]. Such solutions can be used to remove chemical and biological contaminants from wastewater, while concurrently growing biomass for biofuel production, thus proving to be more viable from the economic and technological standpoint [252]. The use of wastewater as a growth medium directly reduces the costs of supplying water and nutrients necessary for the algae to grow at an efficient rate [253]. Research so far has shown that high CO₂ levels in wastewater promote microalgal growth, thus directly stimulating faster degradation of pollutants [254]. In systems where algae are grown in saltwater, the introduction of wastewater also serves to balance the molecular ratio of carbon, nitrogen, and phosphorus (C:N:P = 106:16:1), known as the Redfield ratio [255].

In light of the widely discussed effects of greenhouse gas emissions, integrated systems capable of reducing gas pollutant levels in the air, while simultaneously harvesting biomass and recovering energy, have attracted much interest [252]. One of the most promising and prospective avenues of evolving such systems lies in using microalgal biomass to remove pollutants from waste gases, mainly CO₂, NO₂, and SO₂ [28,256]. Research to date has shown that intensive microalgae cultivation requires a supply of 1.83 kg CO₂ per 1.0 kg of the grown dry matter, which is why low carbon dioxide concentrations in the growth medium often present a bottleneck that impedes rapid biomass growth [200]. Therefore, additional CO₂ needs to be loaded into the photobioreactor by increasing saturation or enriching the culture with leachate from the digesters [29]. Some promising studies on carbon dioxide fixation in algae cultivation systems indicate that the technology may potentially be used to lower CO₂ emissions [28,29].

One advantage of intensive algae production systems is that microalgal biomass can be grown in both freshwater and saltwater media. Kuei-Ling and Jo-Shu (2012) examined *Chlorella vulgaris* ESP-31 growth in freshwater using a modified Bristol's medium and MBL medium, producing biomass concentrations of 2.0–5.0 g dry matter·dm^{−3} for both media [203]. In another study involving *Nannochloropsis salina* CCAP849 grown in saltwater and F/2 medium, Beacham et al. (2015) obtained a final microalgal cell concentration of 7×10^7 cell·cm^{−3} [257]. Unlike terrestrial plants, microalgae do not require fertile farmland to thrive [242,258] and can live, effectively photosynthesize, and build biomass in various climate conditions [238].

Eutrophic and degraded water bodies can be used as another promising source of microalgal biomass [259,260]. Extracting microalgae from such reservoirs leads to a direct improvement in water quality [240,261]. Microalgae blooms, particularly cyanobacteria blooms, pose a threat to regions attractive to tourists and disrupt the basic processes of natural water bodies [262]. For example, Lake Taihu in China, a source of potable water for over two million people, has been repeatedly struck by cyanobacteria blooms since 2007, impacting water quality and posing a technological challenge concerning water treatment [263]. Some researchers have attempted to use microalgae from Lake Taihu as an organic substrate for biogas production [263,264]. Microalgal blooms, most of which are cyanobacteria blooms, are increasingly occurring in water bodies worldwide. Lake Chaohu and Lake Dianchi are among the reservoirs that regularly experience algal blooms [265].

Controlled cultivation of microalgae in eutrophic sea waters has been shown to directly lower biogenic compound concentration in the water and reduce the likelihood of marine life loss. Thus, it can be viewed as a method of revegetation used to improve reservoir condition [253]. Some of the associated issues were addressed in a research program launched by the present authors, which in large part aimed to assess the potential of incorporating microalgal biomass sourced from the Lagoon of Wisła and microalgae sourced from the Puck Bay into methane fermentation processes [266–268]. The analysis of the microalgae sourced from the Lagoon of Wisła showed a taxonomically differentiated biomass undergoing season-to-season changes. Bacillariophyceae species prevailed in the spring months from April to May and in the autumn months from October to November. From June to September, the Cyanoprokaryota division species were the most populous, with Chlorophyta and Dinophyceae as the subdominant groups [262]. It was shown that the time of microalgae extraction from Lagoon of Wisła waters had a significant effect on the organic compound concentration in phytoplankton dry matter. The lowest concentrations were recorded for the Bacillariophyceae-dominant period, whereas the highest ones were correlated with Cyanoprokaryota and Chlorophyta presence [262]. Respirometric analyses showed that the technological performance of the methane fermentation process was the highest in the variants utilizing algal biomass extracted between June and September (i.e., rich in Cyanoprokaryota and, to a lesser extent, Chlorophyta) loaded into model digesters. Biogas yields within this period ranged between 389.07 ± 8.21 and 420.95 ± 0.95 cm³·g dry matter^{−1} [262].

Microalgae can be grown in water sourced from natural reservoirs (with a high content of biogenic substances), as well as in liquid waste and wastewater of various compositions. The use of such culture media not only leads to increased biomass productivity but can also deliver positive

environmental outcomes. Microalgae employed in a photobioreactor with a scrubber allowed for a 60–90% reduction in nitrogen content and 70–100% reduction in phosphorus content in an effluent from manure condensation [269]. Microalgae-based technological systems also offer the advantage of pesticide-free cultivation, which significantly reduces the risk of secondary environmental pollution [238]. Characteristics of microalgal biomass culture systems are collated in Table 11.

Table 11. Characteristics of microalgal biomass culture systems—summary.

Advantages/Disadvantages of Microalgal Biomass Production and Use			Ref.
Culture efficiency		4–100 tons of dry microalgal biomass·ha ⁻¹ ·year ⁻¹ , e.g., <i>Tetraselmis suecica</i> 36 tons of dry microalgal biomass·ha ⁻¹ ·year ⁻¹ (for comparison, soybean production efficiency is at 2.6 tons of beans·ha ⁻¹ ·year ⁻¹)	[235,243,245]
Culture medium	Fresh water	2.0–5.0 g weight·dm ⁻³ using <i>Chlorella vulgaris</i> ESP-31 strain and a modified Bristol medium or MBL	[203]
	Salt water	7 × 10 ⁷ cells of microalgae·cm ⁻³ using <i>Nannochloropsis salina</i> CCAP849 strain and F/2 culture medium	[257]
High photosynthetic efficiency		4–10% (0.5–2.2% in the case of higher plants)	[235,242]
Protein concentration in dry weight		20–60%	[250]
Demand for CO ₂ during culture (a factor impairing high biomass growth)		ca. 1.83 kg CO ₂ ·kg ⁻¹ of dry biomass grown	[200]
Oxidation	Oxygen release during fermentation	1.50–1.92 kg O ₂ ·kg ⁻¹ of the produced biomass	[248,249]
	Oxidation rate during degradation of organic pollutants	0.48–1.85 kg O ₂ ·m ⁻³ ·day ⁻¹	
Redfield's ratio in wastewater-based systems		C:N:P = 106:16:1	[255]
Bio-oil production		19 m ³ ·ha ⁻¹ ·year ⁻¹ (for comparison, 6.1 m ³ ·ha ⁻¹ ·year ⁻¹ from oil palm plantation, 4.3 m ³ ·ha ⁻¹ ·year ⁻¹ from sugar cane, 2.4 m ³ ·ha ⁻¹ ·year ⁻¹ from maize, and 0.5 m ³ ·ha ⁻¹ ·year ⁻¹ from soybean)	[91,241]
Biogas production		389.07 ± 8.21–420.95 ± 0.95 cm ³ ·g dry matter ⁻¹ (with dominating Cyanoprokaryota and subdominating Chlorophyta)	[262]
Use of biogenic compounds		Reduction of nitrogen compound concentration by 60–90% and phosphorus compound concentration by 70–100% in the effluent from manure concentration	[269]

The reservations and controversies surrounding microalgae production/utilization technology mostly relate to the identified investment, technological, and operational barriers to implementation. Such barriers directly impact the costs of biomass cultivation, thickening, and separation. Another dissuading factor is the financial burden connected with converting the biomass into valuable end products [238,239]. The investment and operating costs intrinsic to microalgal cultivation are

several times higher (more than tenfold in some cases) than the costs of extracting lignocellulosic biomass [246,270]. As such, the priority task of commercial enterprises and research groups is to increase the cost-effectiveness of such systems [236]. Furthermore, operating microalgae production installations and converting biomass into other products are still subject to many technological hurdles [258]. Gouveia (2011) noted the multiple deficiencies of algae cultivation methods, pointing to the recurring problems with growing microalgae in photobioreactors, i.e., biofilm build-up on photobioreactor walls, blockage of light sources by the growing culture, high oxygen concentrations, and accumulation of compounds toxic to microalgae cells [271]. Other authors also highlighted the importance of these technological problems [60,272,273].

In order to obtain economically profitable, pure cultures and metabolites of microalgae, it is necessary to employ complex substrate compositions, containing nitrogen, phosphorus, iron, silicon, vitamins, and microelements [240,258]. Operators of intensive microalgae production systems face the major technological challenge of ensuring proper composition of the growth medium and monitoring its quality throughout cultivation. The choice of growth medium depends on the tested microalgae species, as well as on the desired product of cultivation. For example, *Nannochloropsis oceanica* cultivated for biofuel production is grown on BG-11 medium, at 2% CO₂ (v/v), with an artificial light intensity of 80–100 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a temperature of 25 °C [274]. In contrast, *Cryptocodinium cohnii* microalgae grown to produce omega-3 acids need glucose as a source of carbon, yeast extract as a source of nitrogen, a temperature of 27 °C, dark conditions, and oxygen levels of more than 30% [202].

Improper operation of microalgal biomass production systems may lead to problematic environmental pollution with undigested nutrients. This phenomenon causes adverse changes in the functioning and structure of aquatic ecosystems, leading to accelerated eutrophication. The problem stems in large part from bioreactors being fed with an imbalanced nutrient load. The discharge of effluent rich in excess nutrients into natural reservoirs may result in acidification and water pollution, which in turn lead to ecotoxicity, eutrophication, and degradation [261,275].

Other disadvantages of microalgal biomass technologies relate to the potential competition of algae with food crops and industrial crops, land use and the change thereof, and negative effects on biodiversity [258]. Researchers also pointed to potential disruption of natural aquatic ecosystems [276], ozone depletion [277], and structural restrictions on the market's operation [258]. Additionally, genetically modified microalgae used for cultivation may proliferate in the wild and produce various mutations, including ones detrimental to the environment [261]. The lack of legislative/legal measures and incentives, such as subsidies and tax credits, also presents a barrier to the widespread take-up of microalgae-based technologies, including those relevant to biofuel production [276]. The major advantages and disadvantages of algae and algae-derived fuels are presented in Table 12 [13].

Table 12. Major advantages and disadvantages of algae and algae fuels.

Advantages	Disadvantages
Renewable, sustainable, effective, and environmentally friendly biofuel	Incomplete renewable energy resource for biofuels with respect to complete life cycle assessment
Transition to low-carbon economy, i.e., from hydrocarbons to carbohydrate, protein, and lipid resources	Insecurity of algae feedstock supply, regional and seasonal availability, and local energy supply
No competition or lower risk of competition with feeds and foods	Lack of global monitoring and control of algae fuel production with certification of origin and source
Energy security, diversification of fuel supply	Lack of established transparent policy frameworks and instruments (subsidies, mandates and tax credit incentives)

Table 12. Cont.

Advantages	Disadvantages
Enormous greenhouse gas uptake and superior CO ₂ capture and sequestration with extra oxygen release while growing	Possible competition with edible algae and biomaterials production
Conservation of fossil fuels	Utilization of fossil fuels during algae processing
Rural revitalization and social benefits with creation of new jobs and income	High production costs for growing, harvesting, collecting, transporting, storing, and pretreating, as well as the low cost-effectiveness with high initial capital investment
Mitigation of negative effects of spiking crude oil prices and reduced dependency on foreign oil imports	The “biofuel only” production approach is not commercially viable
High energy conversion efficiency by photosynthesis	Damage to natural ecosystems (water, soil, biodiversity conservation, eutrophication, pollution)
High productivity with rapid growth rate and high growing yield	Disruption of the ecological balance in the already stressed lakes, ponds, seas, and oceans
Herbicide or pesticide use is not recommended during cultivation	Harmful algal blooms in global waters
Easily cultured and readily and rapidly bioengineered	Odor, potentially dangerous emissions (Cl, CH ₄ , CO ₂ , SO _x , NO _x , toxic trace elements), ozone depletion, and leaching of hazardous components during disposal and processing
Use of oceans, seas, ponds, and low-productive, degraded and contaminated nonarable lands, can grow even in industrial, municipal, and agricultural wastewaters	Utilization of arable land or land-use changes
Readily adaptable to a wide range of climatic conditions	Use of genetically modified organisms in algae cultivation and in production of biofuels
Reclamation of degraded and contaminated areas and ponds	Unclear utilization of waste products
Prevents eutrophication and pollution in aquatic ecosystems	Health problems due to neurotoxic properties of some algae
Highly biodegradable resource, quick to bioremediate	Technological problems during thermochemical processing (separation, agglomeration, deposit formation, slagging, fouling, corrosion, erosion)
Reduction of algae residues and waste	Restrictions on direct combustion and gasification of algae
Plentiful and relatively cheap resource for production of biochemicals, sorbents, fertilizers, building materials, synthesis of some minerals, and recovery of certain elements and compounds	Lack of accepted terminology, methodologies, standards, and classification and certification systems
High levels of volatiles, Au, B, Br, Ca, I, Mg, P, Ti, carbohydrates, proteins, lipids, structural organic components, extractives, water-soluble nutrient elements	Insufficient knowledge for the assessment and validation; variability of composition, properties, and quality
Low values of C and some trace elements	Algae cultivation occasionally requires high volumes of nutrient-rich water or fertilizers
Great reactivity and low initial ignition and combustion temperatures during conversion	Use of extra water during algae processing
Reduction of some hazardous emissions (CO ₂ , SO _x , NO _x , toxic trace elements) by capture and storage of toxic components in ash	Limited practical experience in biofuel production

5. Conclusions

Pushing forward the development and widespread implementation of clean, effective, and renewable energy technologies represents an ongoing challenge for scientists, as well as a priority issue for operators and administrators of energy systems. There is a widespread perception that this objective can be partly achieved by stimulating the development of unconventional energy generation methods that employ biomass of various characteristics and from various sources. However, this prevailing view is contested by some studies. Mismanagement of traditional energy crop reserves may actually lead to increased greenhouse gas emissions. Other analyses have shown that intensive use of farmland to produce biofuel crops may lead to decreased global food supply and a significant rise in food prices.

Therefore, there is a real need to seek alternative sources of biomass, which would be both commercially and environmentally viable. Algae possess very high photosynthetic efficiency, can rapidly build biomass, are resistant to various contaminants, and can be grown on land that is unsuitable for other purposes. Given these considerations, algae may provide a viable alternative to traditional energy crops. At present, the road to large-scale implementation of technological solutions for the production and use of microalgal biomass is fraught with many economic, technological, and legal difficulties. Unsuitable climate conditions are also a frequent impediment. This means that any microalgae culture facilities should employ technologies that ensure the proper thermal and light conditions—crucial factors in microalgal growth. However, introducing such solutions greatly escalates the investment and operating costs of the technology.

In view of the above, there is a need for solutions that improve the commercial viability of technologies for producing and exploiting microalgal biomass. One of the prospective avenues of improvement is developing and implementing technological solutions that incorporate waste substrates into the growth medium. Sites of anaerobic reactor exploitation can serve as technologically and commercially viable locations for microalgal biomass production, given the supply of ready-to-use biogenic compounds in post-fermentation effluent and carbon dioxide from combusted biogas. Additionally, such installations can provide heat during the cold season. These concepts are fully validated by the fact that the microalgae cultivation systems used thus far in the temperate climate zone are not particularly effective in terms of technology or economy. Therefore, further exploration of novel and alternative solutions is needed to improve the processes of algal biomass proliferation.

Another argument used to defend and support further research on the subject relates to the requirements on the share of biocomponents in the conventional fuel blend and the reduction of greenhouse gas (GHG) emissions. Both of these standards spur the need to implement new technologies of advanced biofuel production that would support the efficient recovery of bioenergy, as well as to implement effective CO₂ sequestration methods. The objective is to increase the share of renewable energy sources (RES) in the energy mix, which directly translates into challenges for the European Union (EU) Member States.

Microalgae-based systems are also increasingly considered for applications in engineering and environmental protection, especially wastewater treatment, solid waste neutralization, flue gas reduction, and biofuel production. The usefulness of microalgae in environmental technologies and in the production of valuable products (including energy products) mostly stems from their higher photosynthesis efficiency, faster biomass growth, and capacity to use and remove waste substances compared with typical terrestrial vascular plants. It is reasonable to assume that, even with the multitude of commercial applications described herein, microalgae still hold untapped potential for the implementation in biotechnology and environmental engineering. Microalgae possess properties that grant them a competitive advantage over terrestrial plants in terms of commercial applicability. The physiological and biochemical characteristics of microalgae directly result from their high genetic diversity. This makes it possible to select and adapt specific strains for individual applications, including environmental protection and engineering technologies. The implementation of a biorefinery approach with the concept of recovering many products from one operational process affords an

opportunity for the development of technologies based on the use of microalgal biomass. Considering the biorefinery complexity index (BCI) as an indicator of technical and economic risk, one of the most promising is a biorefinery platform based on the transformation of microalgal biomass into fuels, food, dietary and feed supplements, fertilizers, and pharmaceuticals.

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