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Review

Towards Sustainable Energy: Harnessing Microalgae Biofuels for a Greener Future

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Abstract: Bioenergy productions from microalgae have received wide attention recently and have a high potential to replace fossil fuels. Moreover, due to the high photosynthetic efficiency, microalgae mass cultivation and scale-up are believed to efficiently reduce the impact of greenhouse gas emissions. This review article explores the potential of microalgae as a reliable and sustainable source of bioenergy feedstock. The current review article contains an in-depth discussion of the various methods of producing energy using microalgae, viz. algal fuel cell (AFC), microbial fuel cell (MFC), bioethanol and biodiesel, and various other applications. This article discussed the different aspects of AFC and MFC, such as fuel cell configurations, reaction mechanisms at electrodes, reactor design factors affecting the efficiencies, and strategies to enhance the efficiencies. Moreover, microalgae cultivation, value-added compounds (pigments, polysaccharides, unsaturated fatty acids), liquid fuel production, limitations, the global scenario of microalgae biomass-based energy, and significant advancements in this field. In a nutshell, this review serves as a valuable resource for identifying, developing, and harnessing the potential of microalgae as a promising biofuel source.

Keywords: algae; biomass; algal fuel cells; reactor design; biofuel feedstock; value added products

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1. Introduction

Fossil fuels are extensively utilized today despite being unsustainable energy sources, resulting in socio-environmental issues. Synchronizing alternative sources of renewable energy is necessary due to the depletion of these fossil fuels [1,2]. The bio-refinery concept, implemented in various sectors globally (energy transportation, food, chemicals, medical, etc.), is pivotal in achieving socioeconomic and sustainable development. One key technological change required for ethanol or gasoline production from industrial waste is the exploitation of diverse organisms in microbial fuel cells. Bioenergy comprises organic waste from plants or animals as well as resources obtained through natural or man-made transformations [3,4].

Over the last few decades, the world has witnessed increasing energy demands and fossil fuel exploitation. As documented by the International Energy Agency, the global consumption of oil in 2019 amounted to approximately 100 million barrels per day, revealing a staggering demand that significantly contributes to the release of detrimental gases into the atmosphere [2,5]. The combustion of fossil fuels emits substantial quantities of pollutants such as carbon dioxide (CO_2), nitrogen oxides (NO_x), and sulfur oxides (SO_x). For instance, the burning of one liter of diesel fuel generates about 2.7 kg of CO_2, along with varying amounts of NO_x and SO_x [3,6,7]. Furthermore, the emission of particulate matter from fossil fuel combustion poses dire health implications [8,9]. Their research demonstrates a compelling correlation between particulate matter exposure and increased mortality rates due to cardiovascular and respiratory ailments. Moreover, these emissions can disrupt ecosystems, contributing to soil and water pollution and impacting vegetation health, aquatic organisms, and biodiversity. The integration of data from these sources underscores the imperative of shifting to sustainable energy alternatives to mitigate the profound ecological and public health consequences associated with current oil consumption and combustion practices [3]. Current energy insecurity is driven by rapid industrialization reliant on fossil fuels, record-high gasoline production, increased dependence on Middle Eastern oil sources, adverse effects of fossil resources on greenhouse gas emissions, and elevated levels of NO_x, SO_x, and metal particles in the atmosphere [10–12]. The adverse consequences associated with non-renewable energy sources, including climate change and ecosystem disruption, necessitate the pursuit of environmentally benign energy derived from renewable sources [13–15]. By 2030, it is projected that fossil fuel consumption will increase by 36% [16]. As the world's population continues to grow, there is an increasing demand for cleaner and more sustainable energy sources [17]. Since fuel-free devices like electric motors are becoming more prevalent, researchers are looking into new sources of power for industrial and residential use [18].

Due to their excellent solar conversion abilities, microalgae are now widely used in the production of biomass and other high-value products. Due to the high costs associated with algae growth and biomass harvesting, the technique, however, has significant limitations [19]. Microalgae have been suggested as a novel feedstock for biofuels in recent literature, addressing problems with traditional renewable fuel sources in terms of resource distribution and food supply [20]. Aerobic bacteria require oxygen to break down organic molecules, and microalgae use byproducts of microbial activity, such as ammonia and carbon dioxide, to supply this oxygen. With the help of this method, eutrophication can now be used to produce biomass and offers resource efficiency [21,22].

Based on current scientific literature, projections indicate that biodiesel production from microalgae could potentially fix up to 2.5 metric tons of CO_2 per metric ton of microalgal oil produced [23,24]. In contrast, fossil fuel combustion releases approximately 2.3 kg of CO_2 per liter of diesel fuel burned [5,21]. Given that microalgae have the inherent capability to capture CO_2 during their growth phase, the net carbon footprint associated with microalgal biodiesel production demonstrates a remarkable advantage over conventional fossil fuels. A study by Clarens et al. [25] presents a comprehensive comparison, indicating that microalgal biodiesel has the potential to achieve a net CO_2 reduction of 75–200% compared to petroleum diesel. This highlights the crucial role of microalgal biodiesel in achieving both sustainable energy production and environmental goals, presenting a viable pathway towards significantly reducing CO_2 emissions associated with transportation fuels.

A comprehensive understanding of the fatty acid composition in both vegetable oils and microalgal oils derived from various species is pivotal, given their potential roles as dietary supplements and biodiesel feedstocks (Table 1). Vegetable oils are traditionally recognized for their nutritional significance, offering essential fatty acids such as linoleic acid (LA) and alpha-linolenic acid (ALA), although relatively limited in long-chain polyunsaturated fatty acids (LC-PUFAs). On the other hand, microalgal oils exhibit remarkable variability in fatty acid profiles due to the diverse range of species and cultivation conditions. Studies by Mata et al. [26] and Schmid et al. [27] highlight the capacity of specific microalgal strains to produce abundant LC-PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with potential applications as dietary supplements for their well-documented cardiovascular and neurological benefits. Notably, microalgal oils' biodiesel potential is underscored by their capacity to yield substantial amounts of lipid content, as indicated by research by Chisti [28] and Mata et al. [26], aligning with their suitability as renewable and sustainable biodiesel feedstocks. These comparisons between fatty acid compositions across different oil sources emphasize the potential dual benefits of microalgal oils, serving both nutritional and energy-related domains, and warrant further exploration to harness their unique qualities for enhancing human health and contributing to a greener energy landscape.

The development of microalgal biofuels has been extensively studied, but to determine the need for legislative action and potential negative effects from outside factors, a thorough analysis of the economic aspects of production and usage is necessary [29]. The adoption of the Kyoto Protocol in 2002 was a significant step towards achieving global sustainability because it brought much of the research on sustainability into line with its goals [30–32]. The production of biofuels must be increased to meet the demand of the global transportation sector and to replace current vehicles [33]. Even though they only account for 1.9% of the total transportation fuel used worldwide now, projections indicate a threefold increase over the next 20 years [29].

To address unfavourable realities surrounding first- and second-generation biofuels, such as energy balance and food security, and to meet the demand for new renewable energy sources, microalgae, a unicellular, ubiquitous organism, has emerged as a potential feedstock for renewable fuels. Studies have been conducted to reduce methane (CH_4) emissions, a significant contributor to greenhouse gases, by interventions such as methane-utilizing bacteria, blue-green algae (BGA), and *Azolla* in irrigated and transplanted flooded rice fields and can be used as biofertilizers [34]. A system where algae are used as biocatalysts in MFCs is generally referred to as AFCs. The use of algae in a fuel cell is advantageous as it not only produces power but also produces biomass that can be used as feedstock for biofuel. MFCs, using microalgae as biocatalysts in the cathodic chamber, generate high power density at low reactor volume [35].

The aim of the present review is to provide the reader with an overall view of microalgae application with specific emphasis on AFC, MFC, and AFC configuration, as well as reactor design, strategies to enhance voltage/power, cultivation of microalgae, use of microalgae as third generation biofuel production, limitations, and global scenario. This review also provides insight into microalgal bioproducts, such as biorefinery products and fertilizers, and their uses in biotechnology and nanotechnology.

Table 1. Fatty acids composition of vegetable and microalgae oils.

Fatty Acids	SP	CP	CV	CM	PMS	SES	RB	SFL	COC	PNT
Butyric acid (C4:0)	0.12	0.38	0.20	nd	nd	nd	nd	nd	nd	nd
Caproic acid (C6:0)	0.28	4.91	2.77	nd	nd	0.38	nd	nd	0.52	nd
Caprylic acid (C8:0)	3.73	3.80	0.26	nd	nd	4.91	nd	nd	7.6	nd
Capric acid (C10:0)	nd	nd	nd	nd	nd	3.8	nd	nd	5.5	nd
Undecanoic acid (C11:0)	0.58	1.45	1.39	nd	nd	nd	nd	nd	nd	nd
Undecenoic acid (C11:1)	0.89	2.63	2.17	nd	nd	nd	nd	nd	nd	nd
Lauric acid (C12:0)	0.49	1.44	0.87	nd	nd	1.45	nd	0.02	47.7	nd
Lauroleic acid (C12:1)	0.39	0.45	0.41	nd	nd	nd	nd	nd	nd	nd
Tridecanoic acid (C13:0)	0.86	0.82	1.03	nd	nd	nd	nd	nd	nd	nd
Myristic acid (C14:0)	0.23	0.65	0.69	13.89	0.17	2.63	0.39	0.09	19.90	0.04
Pentadecanoic acid (C15:0)	1.53	0.88	1.70	0.67	nd	1.44	nd	nd	nd	nd
Pentadecenoic acid (C15:1)	3.16	1.79	3.53	nd	nd	nd	nd	nd	nd	nd
Palmitic acid (C16:0)	46.07	14.63	14.42	20.48	13.1	0.45	20	6.20	nd	7.50
Palmitoleic acid (C16:1)	1.26	3.7	4.04	38.39	0.12	14.63	0.19	0.12	nd	0.07
Hexadecadienoic acid (C16:2)	3.38	5.44	5.34	nd	nd	nd	nd	nd	nd	nd
Hexadecatrienoic acid (C16:3)	0.14	5.01	4.90	nd	nd	nd	nd	nd	nd	nd
Margaric acid (C17:0)	0.27	0.35	0.12	3.26	0.13	0.82	nd	0.02	nd	0.07
Heptadecenoic acid (C17:1)	0.27	nd	0.27	nd	nd	3.7	nd	nd	nd	nd
Stearic acid (C18:0)	1.41	1.4	1.57	1.8	5.7	0.65	2.10	2.80	2.70	2.10
Oleic acid (C18:1)	5.23	18.05	17.62	1.42	24.9	10.45	42.7	28.00	6.20	71.10
Linoleic acid (C18:2-6)	17.43	12.26	11.97	1.6	54.2	nd	33.10	62.2	1.6	18.20
Υ-linolenic acid (C18:3-6)	8.87	nd	nd	1.7	nd	18.05	nd	nd	nd	nd
α-linolenic acid (C18:3-3)	nd	15.75	15.79	nd	0.12	1.4	0.45	0.16	nd	nd
Arachidic acid (C20:0)	nd	nd	nd	nd	0.47	0.88	nd	0.21	nd	1.01
Docosanoic acid (C22:0)	nd	nd	nd	nd	nd	1.79	nd	nd	nd	nd
References	[36]	[37]	[38]	[39]	[40]	[41]	[27,42]	[43]	[44]	[42]

Note: Data are expressed as percentages of total fatty acid methyl esters (FAMEs); nd means that FAs were not determined. Abbreviations of the samples mean: SP—*Spirulina platensis*; CP—*Chlorella pyrenoidosa*; CV—*Chlorella vulgaris*; CM—*Chaetoceros muelleri*; SAF—safflower; SFL—sunflower; PMS—pumpkin seed; SES—sesame; RB—rice bran; PNT—peanut; and COC—coconut oils.

2. Configuration of an Algae Fuel Cell

Algae fuel cells (AFCs) are electro-biochemical systems with photosynthetic microbes incorporated into the anode and cathode compartments Figure 1. These microorganisms derive energy from photosynthesis by acting as electron donors and producing organic metabolites. The primary objective in configuring AFCs is to enhance power density and achieve optimal performance for the development of a cost-effective system. The most common AFC configurations include single chambers, two chambers, three chambers, linked chambers, and sediment types. In the single-chamber AFC, bacteria and microalgae are cultivated together within a single membrane chamber, where microalgae form a biofilm on the anode. In some cases, a single-chamber AFC may incorporate an air cathode [45,46]. Carbon dioxide is consumed by algae within the same container, while bacterial and algal co-cultures are synergistically cultivated in the single chamber AFC [47]. A conventional AFC typically consists of an anode, a cathode, and an electric wire through which electrons flow. In certain cases, a proton exchange membrane may also be present [48]. The main working principle of an algal fuel cell is the breakdown of organic material at the anode, which produces electrons that move along a cable to the cathode and produce electrical energy [35]. To facilitate the operation of the algal fuel cell, the algae are introduced into the device in the form of a biofilm that adheres to a partially exposed cathode. This strategy is used in microbial fuel cells with a single chamber, as shown in Figure 1. Alternatively, photosynthetic organisms such as microalgae can be utilized in the cathode chamber to facilitate the collection of electron acceptors, biomass, and dissolved oxygen (O_2), thereby assisting in electron reduction at the cathode [49,50]. Single chamber AFCs offer ease of management in laboratory settings compared to alternative configurations. They are simple to operate, cost-effective to scale up, and can be utilized for commercial purposes. Additionally, single-chamber AFCs utilize the oxygen produced simultaneously by heterotrophic and autotrophic species. The MFC device was typically used in an H-type cell with two chambers divided by a proton exchange membrane (PEM, 5.5 cm $\times$ 5.0 cm, Nafion-117) in a typical experiment [51].

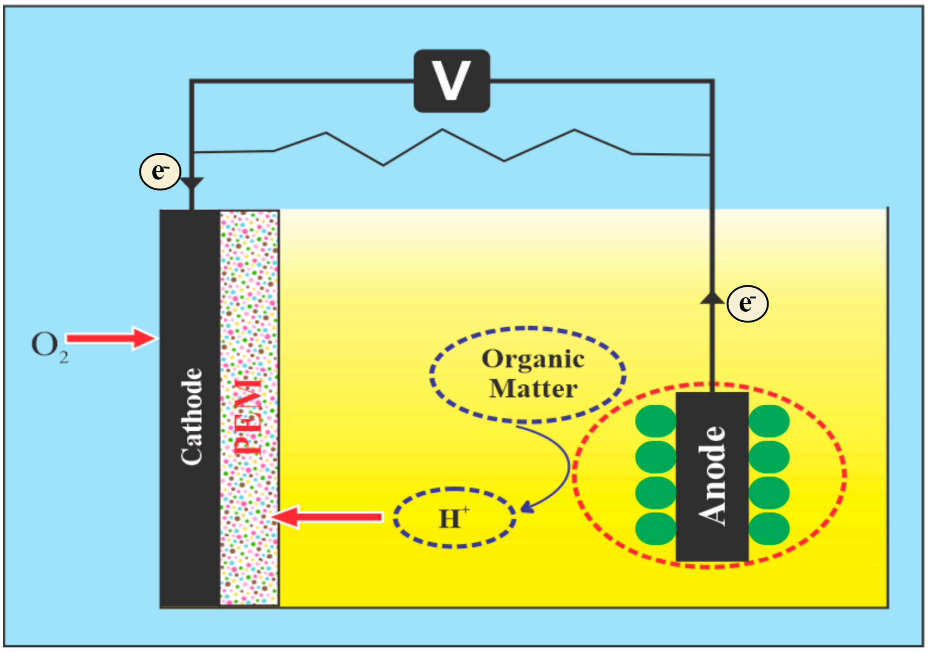


Figure 1. Illustration of a microbial fuel cell with a single chamber [52].

2.1. Oxidation Occurs at the Anode

Microbes have the ability to oxidize organic molecules, represented by the chemical formula $(CH_2O)_n$, through hydrolysis, resulting in the generation of carbon dioxide, H^+

ions, and electrons. In the presence of both algae and bacteria within the anodic and cathodic chambers, the conversion can occur due to the consumption of available materials by the microbial algal fuel cells or the utilization of substrates by the algae [6,52,53].

This process involves the breakdown of organic compounds, leading to the release of carbon dioxide and the production of protons and electrons. The interaction between the microbial and algal populations within the chambers facilitates the oxidation of the organic molecules present in the system [52–54]. The simultaneous presence of bacteria and algae enables the efficient utilization of the available substrates, contributing to the overall performance of the system.

By utilizing the hydrolysis of organic molecules, the microbial algal fuel cells can achieve the oxidation process at the anode. This process is crucial for the overall functioning of the fuel cell system, as it generates essential components such as carbon dioxide, protons, and electrons. To maximize the use of available resources and drive the oxidation reactions, the interaction between the microbial and algal communities is crucial [35,45,53]. Understanding and optimizing the oxidation processes at the anode is essential for enhancing the efficiency and performance of microbial algal fuel cells. Further research in this area will contribute to the development of sustainable and efficient bioenergy systems.

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

2.2. Photosynthesis and Carbon Dioxide Transfer

In alternative systems, algae are housed in separate chambers with distinct anodic and cathodic compartments. The presence of carbon dioxide in these systems promotes algae growth and photosynthetic activity, which improves carbon fixation [6]. The combination of externally supplied carbon dioxide and the release of carbon dioxide from the degradation of organic substances determines the overall carbon dioxide concentration within the system. Following the oxidation of organic molecules, carbon dioxide is removed from the liquid phase by diffusing through the algae's membrane, with the liquid film's resistance acting as a regulatory factor [53]. The algae utilize the delivered carbon dioxide in the presence of light to transform it into organic matter [55]. This generated organic substance is essential for the cellular growth, maintenance, and synthesis of bio-based products within the algal cell [12,56].

These interconnected processes play a vital role in the overall function and productivity of the bio-algal cell system. The availability of carbon dioxide, facilitated by the introduction and utilization within the separate chambers, supports the efficient photosynthetic activities of the algae. The resulting organic matter serves as a critical component for cellular processes, contributing to cell growth and the synthesis of valuable bioproducts [6,53,55,56]. Understanding the dynamics of photosynthesis and carbon dioxide transfer within the bio-algal cell system is crucial for optimizing its performance and productivity. Additional studies in this area will help to advance sustainable bioenergy technologies and use carbon dioxide as a resource.

$$nCO_2 + nH_2O \longrightarrow (CH_2O)_n + nO_2 \text{ (algae in light)}$$

2.3. Cathodic Reduction Process

In most investigations involving AFCs, the algae are typically placed solely in the cathodic chamber [39–41]. Electrons originating from the anode are transferred and combined with H+ ions, which pass through the proton exchange membrane (PEM), and in the presence of oxygen at the cathode, water is produced. The primary source of oxygen in this process is algal photosynthesis. In certain cases, spargers are utilized to introduce oxygen bubbles into the system, ensuring a consistent oxygen concentration [42]. In the case of single-chambered microbial fuel cells, cathodes exposed to air facilitate the availability of oxygen to the cathode biofilm [55,57].

The reduction process occurring at the cathode is a critical step in the overall functioning of AFCs. By harnessing the electrons generated at the anode and combining them with

H+ ions and oxygen, water is produced, which is an essential byproduct of the cathodic reduction. Algal photosynthesis plays a significant role in providing the oxygen necessary for this process. The introduction of spargers ensures the continuous supply of oxygen, maintaining optimal conditions for efficient reduction reactions [57–62].

The performance and effectiveness of AFCs must be improved by comprehending the mechanisms and optimizing the cathodic reduction process. The advancement of cathode design for better oxygen availability and reduction reactions will result from additional research in this field, which will also contribute to the creation of sustainable bioenergy technologies.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

3. Bioactive Organisms: Harnessing the Potential of Microalgae

Microalgae offer a promising solution for bioenergy production, bio-based products, and environmental remediation, making them a versatile option for sustainable bioenergy. This paper provides an overview of the utilization of algal biomass in various applications, including biofuels, food, and environmental purposes. Additionally, valuable co-products and bioactive compounds derived from microalgae are explored. Microalgae, the most diverse group of species on Earth, play a vital role in oxygen production, contributing more than half of the planet's oxygen. The term "microalgae" encompasses various algal kingdoms and divisions, such as Planate, Protozoa, Charophyta, Bacillariophyta, and Chlorophyta [9,63,64]. These organisms, mostly eukaryotes except for cyanobacteria, are microscopic in size and require a microscope for observation.

Microalgae exhibit significantly faster photosynthetic rates compared to terrestrial plants. Table 2 lists the microalgae species used for the extraction of omega-3 fatty acids. Furthermore, the environmental applications of microalgae, including wastewater treatment and carbon dioxide sequestration, are discussed. The production of biomass and photosynthetic rates are influenced by a wide range of factors, including pH, nutrients, salinity, and temperature [65–67]. The potential for wastewater treatment and nitrogen removal of numerous microalgal strains has been demonstrated through laboratory cultivation [68]. However, the transition from laboratory culture to outdoor systems is subject to seasonal variations, temperature fluctuations, and changes in light intensity throughout the day [69]. The symbiotic relationship between microalgae and bacteria has demonstrated the effective removal of organic materials, nutrients, hazardous pollutants, heavy metals, and specific contaminants [15,70,71].

The evolution and limitations of first- and second-generation biofuel feedstocks are discussed in relation to microalgae's unique capabilities for carbon dioxide mitigation in microbial fuel cells. The mechanisms underlying the electron transfer process in microalgae are still not fully understood, and researchers have employed specific inhibitors to inhibit the electron transport chain in these organisms [52]. Microorganisms utilize electrical signals for communication [6], which has been explored through studies on complex communities of autotrophic and heterotrophic organisms, including prokaryotes and eukaryotes, where electrogenic microorganisms exist. Proper strain selection is crucial for maximizing electricity generation in microbial fuel cells [53,72], as shown in Table 3. Efforts have been made to screen and identify suitable microalgae strains, and a recent study aimed to develop a cost-effective and reproducible photosynthetic microalgae fuel cell for testing photosynthetic electrogenic activity in microalgae and cyanobacteria [70]. Promising electrogenic properties have been observed in certain cyanobacteria species, such as *Paulschulzia pseudovolvox* [73].

Further research and exploration of microalgae's bioactive potential, optimization of cultivation conditions and understanding the underlying mechanisms of energy production and communication within microbial communities will pave the way for sustainable bioenergy technologies and environmental applications.

Table 2. The polyunsaturated omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) derived from microalgae.

Species	EPA and DHA in %	References
Nannochloropsis sp.	26.7 EPA + DHA	[74]
Nannochloropsis oceanica	23.4 EPA	[75]
Nannochloropsis salina	~28 EPA	[76]
Pinguiococcus pyrenoidosus	22.03 EPA + DHA	[72]
Thraustochytrium sp.	45.1 EPA + DHA	[72]
Chlorella minutissima	39.9 EPA	[77]
Dunaliella salina	21.4 EPA	[78]
Pavlova viridis	36.0 EPA + DHA	[79]
Pavlova lutheri	41.5 EPA +DHA	[80]
Isocrysis galbana	~28.0 EPA + DHA	[81]

Table 3. Different types of microalgae with oil content involved in biodiesel production.

Species	Oil Content (% wt.)	References
Chlamydomonas sp.	22.7	[82]
Chaetoceros muelleri	13–24	[83]
Parietochloris incise	62	[66]
Tetraselmis tetrathele	25–30	[84]
Nostoc commune	22	[84]
Emiliania huxleyi	43.8	[84]
Chroomonas salina	12–14.5	[84]
Mesotaenium sp.	19–35	[66]
Spirulina Platensis	4–11	[84]
Synechocystis sp.	11	[85]
Nannochloris sp.	25–56	[86]
Neochloris oleoabundans	35–65	[86]
Chlorella sp.	28–53	[86]
Schizochytrium sp.	50–77	[87]

4. Microalgae as Third-Generation Biofuel Feedstock: A Sustainable Solution

Microalgae have received a lot of attention as a potential feedstock for biofuels due to their many advantages over traditional feedstocks made from plant crops and agricultural waste streams. Microalgae can be grown on non-arable land, allaying concerns about competition with food production and increasing the potential for biofuel production [54]. With a wide range of species, from unicellular genera like Chlorella and diatoms to macroalgae, microalgae present a diverse pool of eukaryotic and prokaryotic cells [54,86,88]. Carbohydrate and lipid derivatives derived from microalgae can serve as feedstock for bioethanol production [89].Comparisons have been made between biomass and lipid generation, as well as pollutant removal efficiency, using different cultivation methods such as photobioreactors [90].

First-generation biofuels are made from plant-based materials, such as food crops like corn and sugarcane, whereas biodiesel is made from oils like palm and soybean oil [91]. Microalgae can be either autotrophic, needing only inorganic materials and light as their energy source [89,92], or heterotrophic, needing additional nutrients and organic molecules [28]. These tiny algae species, measured in micrometres, exhibit higher lipid content and faster growth rates than bacteria [88,93]. Despite the vast number of microalgae varieties, only a fraction has been selected for research purposes [12,94,95]. Their rapid harvesting cycle, outperforming traditional crops harvested once or twice a year, offers a significant advantage [96,97].

Various biomass sources, including agricultural and forestry waste as well as aquatic sources, have been proposed for biofuel production, such as biodiesel, bioethanol, biohydrogen, bio-oil, and biogas. However, the environmental impact and limited availability of these biomass sources have raised concerns about sustainability and forest destruction [54,89,98–103]. This has led to a focus on algal biomass as a promising alternative for biofuel production. Microalgae can be converted into various liquid biofuels, such as bioethanol, syngas, cellulosic ethanol, biogas, biodiesel, and bio-oil, through processes like liquefaction and combustion. The potential of microalgae as a biofuel feedstock has attracted the interest of scientists and businesses alike, as it offers a sustainable and independent source of biomass that does not interfere with food production. To support the production of triacylglycerol and carbohydrates for biofuel synthesis, microalgae require light, carbon dioxide, and essential nutrients [21,89,104,105].

However, difficulties still exist, such as the competition for agricultural and water resources, which have been discussed in relation to third-generation microalgae-based biofuels [106–108]. Marine microalgae, such as seaweed, have shown promise for bioethanol production, while biodiesel derived from microalgae has emerged as a viable option [109–113] via transesterification, as shown in Figure 2. Microalgae exhibit better growth performance in various water resources, with higher lipid deposition capacity and biodiesel energy content compared to ethanol [114–118]. Table 3 shows different algal species. The scalability and processing capabilities of microalgae-derived biofuels make them a potential solution for current and future fuel demands. Cultivation of microalgae typically occurs in controlled environments, including open ponds or enclosed reactors called photobioreactors, where nutrient-rich conditions and carbon dioxide supply promote biomass growth [119–121].

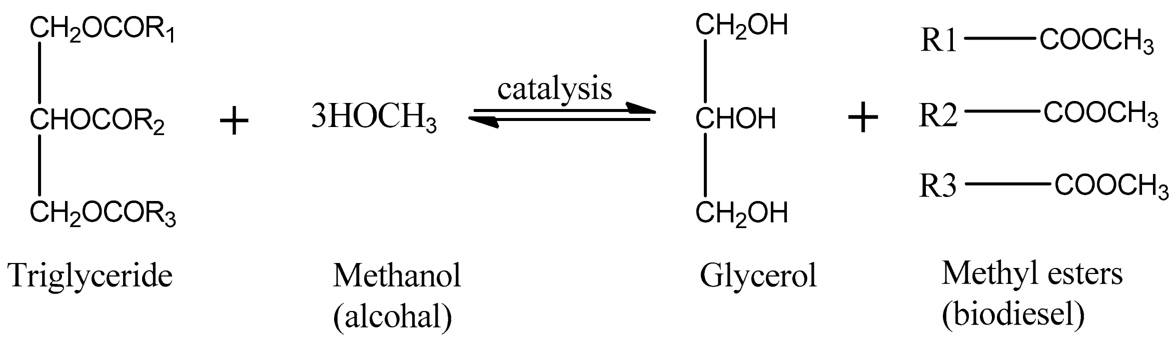


Figure 2. Transesterification reaction mechanism (R1, R2, and R3 are long-chain hydrocarbons, sometimes fatty acid chains).

In conclusion, microalgae present a sustainable solution as a third-generation biofuel feedstock, offering higher biofuel production potential, independence from arable land, and a diverse range of species for cultivation. Microalgae are a promising renewable energy source for a greener future due to their capacity to produce a variety of biofuels, rapid growth, and minimal impact on food production [88,108].

5. Photosynthesis in Microalgae Cells: Harnessing Light Energy

Microalgae have received a lot of attention as a potential feedstock for biofuels due to their many advantages over traditional feedstocks made from plant crops and agricultural waste streams. Microalgae can be grown on non-arable land, allaying concerns about competition with food production and increasing the potential for biofuel production [56,122,123]. With a wide range of species, from unicellular genera like *Chlorella* and diatoms to macroalgae, microalgae present a diverse pool of eukaryotic and prokaryotic cells. Carbohydrate and lipid derivatives derived from microalgae can serve as feedstock for bioethanol and biodiesel production, respectively. Comparisons have been made between biomass and lipid generation, as well as pollutant removal efficiency, using different cultivation methods such as photobioreactors [89,90,124,125].

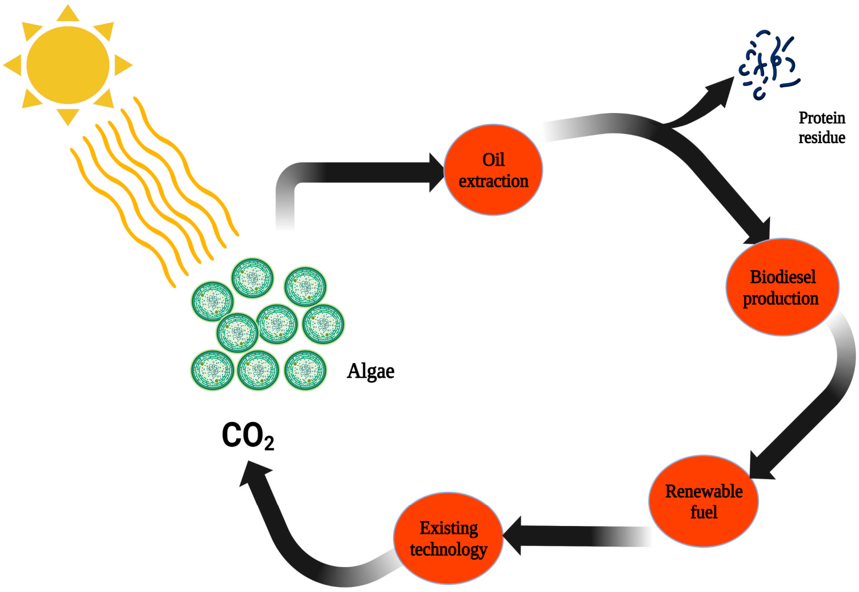
To produce biofuels like biodiesel, bioethanol, biohydrogen, bio-oil, and biogas, a variety of biomass sources have been suggested. These sources include aquatic sources as well as agricultural and forestry waste [54,98–101,126]. However, the negative effects on the environment and the scarcity of these biomass sources have prompted questions about sustainability and the loss of forests [28,102,103]. This has led to a focus on algal biomass as a promising alternative for biofuel production. Microalgae can be converted into various liquid biofuels, such as bioethanol, syngas, cellulosic ethanol, biogas, biodiesel, and bio-oil, through processes like liquefaction and combustion [21]. The potential of microalgae as a biofuel feedstock has attracted the interest of scientists and businesses alike, as it offers a sustainable and independent source of biomass that does not interfere with food production [92]. Microalgae need light, carbon dioxide, and critical nutrients to support the production of triacylglycerol and carbohydrates for biofuel synthesis [21,89].

However, challenges still exist, such as the competition for agricultural and water resources, which have been discussed in relation to third-generation microalgae-based biofuels. Marine microalgae, such as seaweed, have shown promise for bioethanol production, while biodiesel derived from microalgae has emerged as a viable option via transesterification, as shown in Figure 3a,b [106,107,109,127–129]. Microalgae exhibit better growth performance in various water resources, with higher lipid deposition capacity and biodiesel energy content compared to ethanol [130]. Table 4 shows different algal species with corresponding reactor design, electrode material, and voltage/power. The scalability and processing capabilities of microalgae-derived biofuels make them a potential solution for current and future fuel demands. Cultivation of microalgae typically occurs in controlled environments, including open ponds or enclosed reactors called photobioreactors, where nutrient-rich conditions and carbon dioxide supply promote biomass growth [12,28].

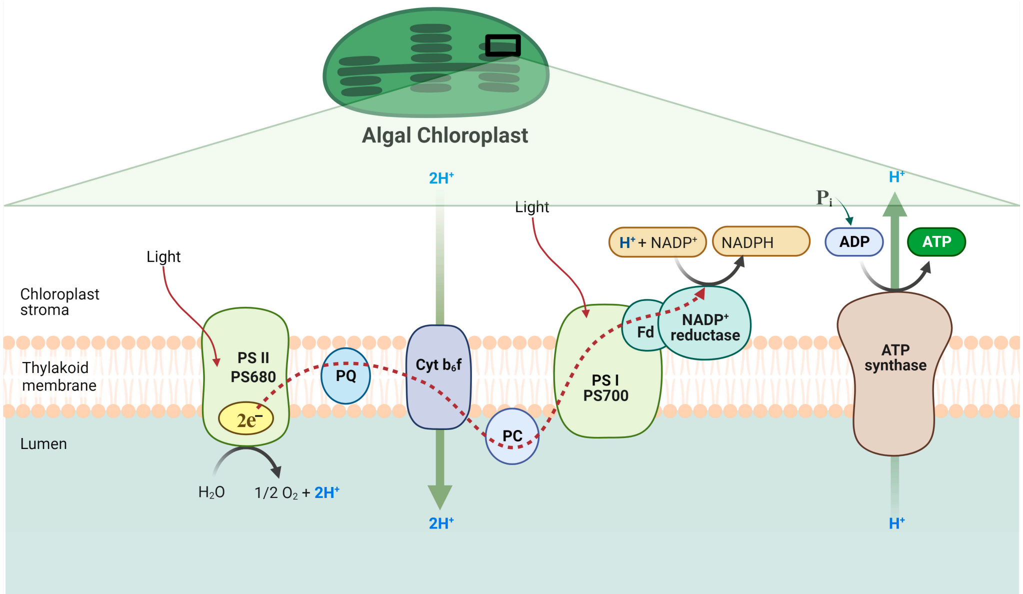
Table 4. Comparing the power densities of various microbial fuel cell configurations [53].

Algal Strain	Power/Voltage	Reactor Type	Electrode
Chlorella pyrenoidosa	6400 mW/m^3	DCMFC/Mixed anaerobic sewage sludge in synthetic WW/BG-11	Carbon felt
Chlorella vulgaris	36.4 mW/m^2	DCMFC/Activated sludge/BG-11	Nickel foam/Graphene
Chlorella vulgaris	123.2 ± 27.5 mW/m^3	Textile wastewater/No catholyte, algal biofilm on air cathode	Carbon fibre brush
Chlorella vulgaris	126 mW/m^3	DCMFC/Municipal Wastewater/BG-11; SCMFC/Real dye	Stainless Steel Mesh
Chlorella vulgaris	3720 mW/m^3	PAMFC/Swine WW/Diluted swine WW	Carbon felt/carbon fibre
Chlorella vulgaris	1108.9 mW/m^3	Bubbling PAMFC/Anaerobic sludge with phosphate buffer supplemented with glucose/BG-11	Carbon felt/Carbon fibre cloth
Mixed Algal Culture	3300 mW/m^3	PAMFC/Anaerobic sludge/Anolyte effluent anaerobic sludge	Carbon brush/Carbon cloth
Mixed Algal Culture	268 mW/m^2	PAMFC/Anaerobic sludge/Anolyte effluent anaerobic sludge	Carbon fibre brush/Carbon cloth
Scenedesmus obliquus	153 mW/m^2	DCMFC/Municipal wastewater/BBM	Plain carbon paper/carbon paper
Scenedesmus obliquus	951 mW/m^3	DCMFC/Municipal wastewater replaced later with BBM/Ferricyanide	Toray carbon paper

In conclusion, microalgae present a sustainable solution as a third-generation biofuel feedstock, offering higher biofuel production potential, independence from arable land, and a diverse range of species for cultivation. Due to their ability to generate a variety of biofuels, rapid growth, and minimal impact on food production, microalgae are a promising renewable energy source for a greener future.



(**a**)



(**b**)

Figure 3. (**a**) An overview of the photosynthesis pathway in algal chloroplasts for biofuel production. (**b**) Overview of photosynthetic pathway in algal chloroplast. The light energy excites the electron in P680 to a higher energy state. This electron gets transported via the membrane to the P700, where it replaces the excited electron in the P700 complex. The second excited electron is then transported through the membrane and ultimately reduces NADP+ to NADPH. The missing electron in P680 is replaced through the hydrolysis of water, which leaves free H^+ in the cell. This is then transported through the ATP synthase to facilitate the conversion of ADP to ATP, the major source of energy for biochemical reactions in the cell.

6. Biomass Pre-Treatment for Microbial Fuel Cells (MFCs): Enhancing Efficiency

Efficient hydrolysis of biomasses like lignocellulose in the anode chamber of MFCs is a challenge for most microorganisms, leading to a low Power Density (PD). The hierarchical structure and strong hydrogen bonding of lignocellulose make it resistant to enzymatic treatment and hinder the release of fermentable sugars. To address these limitations, pre-treatment techniques are necessary to make different reducing sugars more accessible. The effectiveness of pre-treatment methods relies on biomass characterization, and various pre-treatment approaches for microalgae are summarized in Table 5 [131–133]. These pre-treatment techniques play a crucial role in maximizing the recovery of valuable products in the bio-refinery process of MFCs.

Table 5. List of pre-treatments of algal biomass to improve biogas yield [134].

Biomass	Pre-Treatment	Increased Biogas Yield
Chlorella vulgaris	Proteases (86–96% solubilization)	51%
Scenedesmus sp.	Thermal (75 °C for 10 h)	58%
Stigeoclonium sp.	Thermal (130 °C for 15–30 min)	28%
Nitzschia sp	Thermal (130 °C for 15–30 min)	28%
Scenedesmus sp.	Thermal (95 °C for 10 h)	69%
Chlorella sp.	Thermal (70 °C for 30 min)	37–48%
Chlamydomonas reinhardtii	Proteases (86–96% solubilization)	7%
Chlorella sp. and *Scenedesmus* sp.	Chemical (CaO; 4 and 10% *w*/*w*) at 25, 55 and 72 °C	25%
Monoraphidium sp. and *Stigeoclonium* sp.	Mechanical (26.7 KJ/g TS for 30 min)	85%
Scenedesmus sp.	Proteases (30% solubilization)	1.53-fold
Chlorella vulgaris and *Scenedesmus* sp.	Carbohydrases (84% and 36% solubilization)	1.2-fold

6.1. Physical Pre-Treatment for Biomass

Physical pre-treatment methods such as grinding and chipping can reduce particle size and break down the crystallinity of biomass, increasing its biodegradability in MFCs [134]. Particle size has been found to be an important factor in achieving maximum bioenergy output, with a further reduction to particles less than 40 mesh yielding favourable effects on hydrolysis rate and yields [135]. Additionally, irradiation procedures like X-rays and electron beams can be utilized as physical pre-treatment methods for biomass.

6.2. Acid Pre-Treatment for Biomass

Acidic pre-treatment is a commonly used chemical process to enhance the efficiency of enzymatic hydrolysis and energy conversion of lignocellulosic biomass in MFCs. Concentrated mineral acids (CA), dilute mineral acids (DA), and dicarboxylic acids have been employed for lignin-based biomass pre-treatment. Diluted H_2SO_4 pre-treatment has been shown to achieve a high rate of reaction, and it has been used in MFCs for direct electricity generation from maize straw. Diluted sulphuric acid pre-treatment of rice straw has also been investigated, resulting in improved power density in solid-state MFCs [135,136].

6.3. Alkali Pre-Treatment for Biomass

Alkali pre-treatment using base reagents like potassium hydroxide and sodium hydroxide is effective, especially for biomass with low cellulose and lignin content, such as agro-waste [137]. The reaction time for alkali pre-treatment is relatively long, ranging from several hours to a few days. Sodium hydroxide pre-treatment of rice husk has been studied for utilization in solid-state MFCs, resulting in significantly higher power density compared to unprocessed MFCs. Alkali pre-treatment has also been explored for mud (sludge) fueled microbial fuel cells [137–139].

6.4. Enzymatic Pre-Treatment for Biomass

Enzymatic pre-treatment offers a non-toxic, recyclable, and energy-efficient approach for biomass modification. Cellulose-degrading enzymes produced by fungi and bacteria can degrade and break down the polymeric structure of lignocellulosic materials, leading to increased sugar production and improved MFC performance [140]. Various microorganisms such as *Erwinia*, *Bacillus*, *Ruminococcus*, *Clostridium*, *Acetovibrio*, *Microbispora*, *Bacteriodes*, *Cellulomonas*, and *Streptomyces* are capable of producing cellulase enzymes [141]. White-rot fungi have been found to be the most efficient for biological pre-treatment of biomass, as they can decompose lignin and cellulose [135,136]. Brown-rot fungi, on the other hand, primarily feed on cellulose [134]. Enzymes targeting hemicelluloses, such as glucuronidase, acetylesterase, xylanase, xylosidase, galactomannanase, and glucomannanase, also play a role in the breakdown of lignocellulosic materials [142]. However, biological hydrolysis is often slow and presents challenges such as long retention time, contamination control, and space requirements, limiting its commercial viability [136].

7. Methods for Increasing Power Production through Improved Reactor Design

7.1. Reactor Designs and Components

There are three major reactor types used in AFCs: Single Chambered Microbial Fuel Cell (SCMFC), Double Chambered Microbial Fuel Cell (DCMFC), and Microbial Fuel Cell Based on Photosynthetic Microalgae (PAMFC) Figure 4. All three reactor types consist of anode and cathode electrodes for oxidation and reduction, respectively. Double-chamber microbial fuel cells and PAMFCs feature a proton exchange membrane (PEM), while PEMs are used only in some single-chamber microbial cells. In the anode compartment, anolyte is used as the medium, while catholyte is used in the cathode compartment. In SCMFCs, the electrodes are immersed in a single medium [71,121,132].

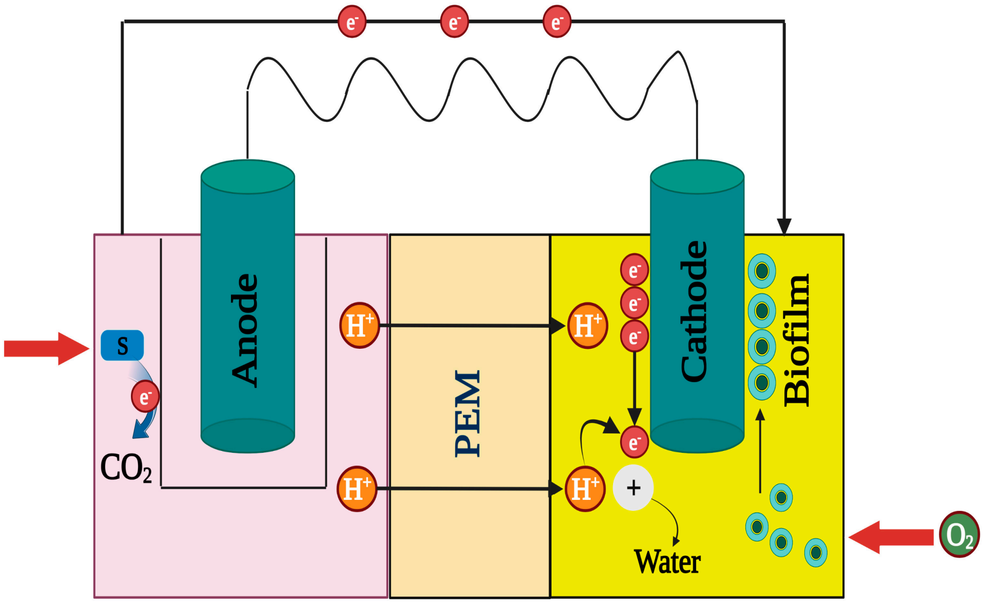


Figure 4. Double chamber microbial fuel cell based on photosynthetic algae, separated by a large proton exchange membrane.

7.2. Reactor Designs

7.2.1. Single-Chambered Reactor

Single-chamber microbial fuel cells typically have a cubic or cylindrical shape [137,138]. Power generation occurs when organic molecules break down and produce electrons, which travel from the anode chamber to the cathode chamber via a wire connected to an external load. In PAMFCs, exposing the cathodes to air promotes the reduction of oxygen produced during photosynthesis, as they act as terminal electron acceptors. This is advantageous as the concentration of dissolved oxygen is a critical factor in AFC performance. When an algal biofilm is used with an air cathode, sufficient oxygen is available, eliminating the need for catalysts. SCMFCs involve the growth of both bacterial and algal cultures in tandem [139].

7.2.2. Double Chambered Reactor

Double chamber microbial fuel cells (DCMFCs) consist of two independent chambers separated by a PEM membrane. This configuration is popular in microalgae assisted MFC experiments due to its efficiency. The anode, cathode, and PEM can be enclosed in one tank or arranged in an H-shape, with the PEM incorporated into the arm. H-shaped DCMFCs have downsides, such as excessive fouling and a small membrane area, which reduce power production. However, they are suitable for small-scale experiments due to low capital costs [121,143].

7.3. Reactor Components

7.3.1. Electrodes

The composition of electrodes significantly affects the effectiveness of algae microbial fuel cells. Desirable characteristics of good electrodes include high electrical conductivity, large surface area, biocompatibility, corrosion resistance, low resistance, mechanical resilience, and low production cost [57,71,140]. Porous ceramics and modified carbons such as activated carbon, carbon felt graphite, and carbon black are commonly used as electrode materials. Modifications such as carboxyl grafting, graphene modification, nitrogen doping, fluorine doping, acetate pre-treatment, and others improve electron transfer and create reaction sites, resulting in higher substrate oxidation and increased energy production in the fuel cell [82,143–145]. Understanding reactor design in bacteria based MFCs can also aid AFC design, as both systems face challenges related to biofilm growth on the electrodes, which reduces electron transport and power production. Porosity and surface roughness of the electrodes are important qualities to ensure uniform distribution of the cathode biofilm. Flat electrodes do not allow biofilm growth, while highly porous electrodes can reduce mass transfer but help prevent biofilm detachment [146–149]. Biofilm growth on the anode facilitates electron transfer but can hinder power production if the biofilm resistance increases significantly. In two-chambered reactors, the anode is submerged in wastewater containing a minimal number of bacteria necessary to generate a biofilm favourable for energy generation [144,150,151].

7.3.2. Proton Exchange Membrane

In double-chambered microalgae fuel cells, after oxidation occurs at the anode, electrons pass through a wire, while protons travel through a PEM from the anodic chamber to the cathodic chamber. The PEM's primary function is to transfer H^+ ions from the anode to the cathode, where they combine with dissolved oxygen to produce water. The PEM needs to be compatible with both anodic and cathodic media since it acts as a barrier between the chambers. Desired qualities include mechanical strength, high ionic conductivity, and support for steady ion transfer. PEM materials that can absorb water are used to ensure stability. Commonly used materials include hydrogels [74,144,152,153] or polymers such as cross-linked gel polystyrene [53]. Combining polyvinyl alcohol hydrogel with clay has been attempted to enhance water uptake and proton conductivity, resulting in improved power density in modified PEMs [154].

7.3.3. Carbon Dioxide Chamber

Microalgae utilize carbon dioxide during photosynthesis to produce glucose for metabolic activities and growth [155]. Carbon dioxide utilization is one of the main advantages of algae fuel cells (AFCs) compared to typical microbial fuel cells. To achieve carbon sequestration, a gas outlet pipe is connected from the anode chamber to the microalgae-filled cathode chamber, where the organism's growth occurs [35,62]. Some reactor designs have a sparger inside the cathode chamber to provide carbon dioxide.

8. Factors That Influence Microalgae Growth

8.1. Light

Light is the most crucial factor for the photosynthetic growth of microalgae. It influences the cellular composition of microalgae through processes like photo adaptation or photo acclimation. Adequate light availability is essential as it serves as the substrate for photosynthesis. The intensity of light, known as the compensation point, determines the transition from cellular respiration to photosynthesis [76,107]. At this point, the rate of photosynthesis reaches its maximum, known as photo limitation. The amount of light available is measured in photon flux density (E or mol m^2 s) or photon absorption rates (E or mol kg cells).

8.2. Carbon Dioxide

Microalgae require carbon dioxide for photosynthesis, which can be supplied in the form of bicarbonate or by direct infusion into the air. Ambient air alone is usually insufficient for intensive microalgae production. Before microalgae can utilize carbon dioxide, it needs to be dissolved, and the dissolution process depends on pH levels. An ionic-strength microenvironment is ideal for microalgae growth, leading to the presence of carbon dioxide and carbonate ions as the main soluble carbon forms [6,80].

8.3. Temperature

The temperature in the closed system, particularly in the photobioreactor, increases with solar energy and air temperatures [65]. Heat is transferred to the medium through natural or induced convection, radiation, and direct and diffuse solar radiation [76]. For optimal growth of phototrophic microalgae, the temperature should be maintained between 15 and 30 °C (60 and 80 °F).

8.4. Nutrients

Nitrogen is the most critical element for structural and operational proteins, second only to carbon. Microalgae exhibit similar growth rates regardless of the nitrogen source (urea, nitrite, or nitrate). Studies have shown that nitrogen deficiency leads to increased fat production and storage. Phosphorus, as a macronutrient, plays a vital role in cellular metabolism. Phosphorus deprivation may cause pigment accumulation in some microalgae, although its impact is less significant than that of nitrogen deficiency. Other nutrient sources, such as farmyard compost and agricultural wastewater, are being explored for microalgae cultivation. For instance, *Chlorella vulgaris* was grown using chicken compost as a nutrient source, and its growth and fatty acid content were evaluated [9,110,156]. High fertilizer concentrations significantly affected biomass productivity, and the total lipid content ranged from 26% to 37%, with an average of 31%. This study demonstrated that chicken compost can serve as a rich alternative nutrient source for microalgae growth.

8.5. Culture Medium

The quality of the culture medium can affect contamination in microalgae cultures and make sterilization challenging. The water used in the medium is particularly important, and seawater with unpredictable pollutant levels poses a significant challenge. The seawater may contain vitamins, chelating agents, buffers, soil extracts, and other components that contribute to the culture medium [12,112].

9. Global Microalgae Oil Market

The application of microalgae oil in the food and beverage sector is expected to hold a significant share of the market. Microalgae oil is rich in antioxidants, proteins, and essential omega-3 fatty acids, making it highly beneficial for human health [77]. Increasing consumer preference for nutritious foods and pharmaceuticals, along with growing health and fitness awareness, is driving market growth. The market is segmented into macroalgae, and microalgae based on product categories, with macroalgae occupying the largest market share due to its widespread use in various food products. Macroalgae also have higher fibre content compared to microalgae. The feed grade segment is predicted to witness growth during the forecast period as algal oil serves as a nutrient-rich alternative to grain feed. Additionally, microalgae cultivation requires less water and land, providing a significant advantage [5].

The global microalgae oil market was valued at USD 1.63 billion in 2020. It is projected to grow at a compound annual growth rate (CAGR) of 4% between 2022 and 2027, reaching a market value of approximately USD 2.07 billion by 2026 [5].

10. Global Microalgae Market 2020–2026

The global microalgae market is projected to grow at a compound annual growth rate (CAGR) of 6.5% during the forecast period of 2020–2026 [157]. With the increasing demand for superfoods and the rising health and wellness trend, both macroalgal and microalgal cuisines, along with advancements in microalgal technology for application development, are driving market expansion, as shown in Figure 5. In the United States, the microalgae market is expected to reach USD 286.1 million in 2021, holding a 29.14% market share globally. China, the second-largest economy, is anticipated to grow at a CAGR of 6.5% during the research period, reaching an estimated market size of USD 253.5 million by 2026. The current focus on sustainability has brought algae biotechnology back into the spotlight after years of neglect. The global algae biotechnology market, projected to be worth USD 939.4 million in 2020, is now expected to expand to USD 1.3 billion by 2026, with a CAGR of 5.3% throughout the study period. One segment of the market, *Spirulina*, is anticipated to grow at a CAGR of 6.2% and reach USD 577.8 million by the end of the research period. Algae, including *Chlorella* sp. and *Spirulina* sp., are increasingly being used for culinary and nutritional purposes. *Spirulina* pills, marketed as superfoods, are gaining prominence in various health and energy products [157].

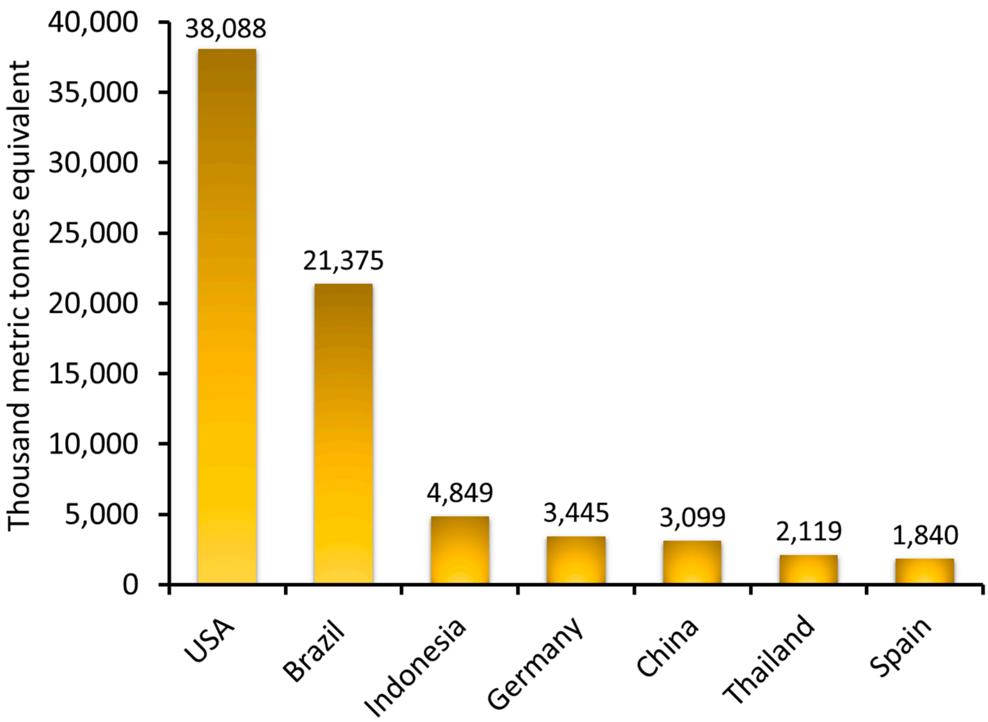


Figure 5. Leading countries in biodiesel production.

11. Worldwide Technologies Used for Microalgae Biofuel Production by Companies

When comparing the financial feasibility of biofuel production to that of fossil fuels, traditional biofuels often have higher processing costs and, therefore, less competitive market prices [126]. For instance, soybean-based biodiesel was approximately 20% more expensive to produce than diesel in 2005, while ethanol production was about 5% costlier than gasoline [154]. The increase in fossil fuel prices since then (around 30%) has made biofuels relatively more affordable, but fossil fuels still dominate the market [22,110,129]. Government subsidies have allowed certain biofuels to enter the energy market through mandates and tax credit programs. A notable example is Brazil, where ethanol produced from sugarcane has gained significant market presence [157]. Market or output-based costs do not fully capture the potential non-market benefits associated with biofuel production and use. Considering these benefits, such as lower environmental footprint, improved fuel accessibility, and national/regional energy independence, subsidies can enhance the quantity and price efficiency of biofuels [156,158–160]. However, it is important to critically assess the assumptions regarding the existence and practical significance of these benefits.

Large-scale microalgal growth is attractive to biofuel producers due to its environmental friendliness, utilization of global technologies, reduction in fossil fuel dependency, and minimal impact on food crops. Carbon dioxide, nitrous oxide, fluorinated gases, and methane are the primary greenhouse gases, with carbon dioxide contributing to 77% of global emissions. Crude oil and production processes account for 65% of carbon dioxide emissions [103,161,162]. The potential impact of biofuels on food prices is higher than that on energy prices, particularly for first-generation feedstocks [89,105]. Maize and corn products, for example, have experienced price increases due to their allocation for ethanol production, resulting in a 40% rise in ethanol-related costs. This has implications for grain and livestock prices, leading to a 5–15% increase [161].

Concerns have been raised regarding the impact of biofuel production on surface and groundwater resources. With a growing global population and limited agricultural land resources, traditional biofuels may not be sustainable, leading to a projected 45% increase in farmland consumption by 2020 [21,89]. However, this increase will only meet a small portion of petroleum demand. The pressure on farmers to convert crops for biofuel production has already affected food prices due to increased biofuel demand. This competition for agricultural land has also contributed to significant palm oil deforestation in Southeast Asia, resulting in decreased carbon storage and biodiversity. Second-generation feedstocks can further exacerbate food and grazing land competition, particularly in poorer urbanized areas [163,164]. The true impact of increased biofuel use on food prices is difficult to evaluate due to various factors, including direct crop competition and indirect competition for agricultural resources. Biofuels are not yet widely adopted in most countries [21,89,105].

12. Application of Nanomaterials in Microalgae Cultivation

The collection of microalgae and the extensive use of arable land are significant challenges in microalgae cultivation. Nanomaterials, which explore nanometre-sized objects with distinct characteristics and functions due to their small size, offer a promising approach to creating, utilizing, and understanding substantially different material structures, systems, and processes. Nanotechnology, with its effective physiochemical and crystallographic properties, is a field of great interest. Nanoparticles exhibit unique heating, biological, optical, electrical, chemical, and physical qualities compared to their bulk-scale counterparts [165,166].

Nanoparticles find applications in various fields, such as medical treatments, efficient power generation in solar and fuel cells, and pollution control in water and air filters. They can also serve as catalysts in conventional manufacturing processes, replacing toxic substances. Functionalized nanomaterials have been developed to enhance biofuel production, including nickel-based, hydrophobic-based, gold-based, and amino-based catalysts. Adding nanoparticles to the cultivation medium can improve the efficiency of these catalysts [23,167]. However, the characteristics of these nanomaterials, such as excessive metal content (e.g., iron) and their competition for nutrients with microalgae cells, can induce stress on microalgae, affecting lipid formation. Nanoparticles are generated catalytically in a liquid medium using reducing and stabilizing substances, such as potassium bitartrate, methoxy polyethylene glycol, sodium dodecyl benzyl sulfate, and sodium borohydride. These nanoparticles have demonstrated remarkable efficiency in lipid extraction and recovery, replacing traditional chemicals like methanol, ethanol, hexane, petroleum ether, and chloroform [9,167,168].

The advantages of nanomaterials include preventing microalgae cell death and accelerating the re-cultivation process after cell removal [159,169]. However, physical, and chemical techniques used in both systems, such as attrition and pyrolysis, have drawbacks like environmental impact, labour-intensive processes, and high costs. Nanoparticles have high surface energy, attracting atoms and molecules and altering their surface characteristics. Therefore, they cannot exist in their native state in the environment. By creating a slightly stressful environment that allows lipid accumulation without damaging the cells, these unique nanoparticles enable microalgae to perform photosynthesis. For instance, iron and magnesium nanoparticles have been used in several experiments as nutrient supplements in the growth medium. Iron nanoparticles generate reactive oxygen species through a Fenton-type reaction, causing oxidative stress in microalgae cells. Conversely, it has been observed that a higher concentration of TiO2 nanoparticles can enhance cell vitality in the presence of light [163,169,170]. Additionally, Mg-amino clay nanoparticles have shown positive effects on the growth of Chlorella sp. The amino clay nanoparticles, bonded with metal cations like Mg^{2+}, Fe^{3+}, Ca^{2+}, and Ag^{3+}, promote growth and can also enhance photosynthesis and reduce glycerol intake in *C. vulgaris* heterotrophic cultures [64,171,172]. These nanoparticles have also been used as enzyme immobilizers for biodiesel production, resulting in significant biodiesel conversion rates [173]. Metallic nanoparticles can be produced from microalgal biomass in a one-step process by adding an aqueous solution of metallic salts to the cells while they are still in their growth conditions [173].

Once created, nanoparticles are dispersed into the culture medium while still enclosed in a matrix, forming colloids. These colloids settle in the photobioreactor due to their significant weight. If necessary, repeated cycles of nanoparticle production can be carried out by adding a fresh growth medium. Microalgae can also biosynthesize nanoparticles when trapped inside organic vesicles. Various analytical techniques, including scanning electron microscopy, FTIR spectroscopy, TEM dynamic light scattering, UV-Vis spectroscopy, powder X-ray diffractometry (XRD), atomic force microscopy, and X-ray photoelectron spectroscopy, can be used to characterize the morphology of metal nanoparticles [94,174]. These advancements have increased awareness of the importance of developing non-toxic and environmentally friendly methods for nanoparticle assembly and synthesis.

13. Factors Affecting Microalgae Growth and Their Impact on Fuel Cell

The attached microalgal biomass has been found to effectively remediate nitrogen-rich wastewater. Incorporating nutrients into microalgal biomass has become a common strategy for wastewater bioremediation. In one study, a fluidized bed bioreactor was used to cultivate attached microalgal biomass on polyurethane foam support material while treating real wastewater from the chemical fertilizer manufacturing industry. Under optimal conditions, including a light intensity of 216 mol/m^2 s and a carbon dioxide concentration of 9.1%, complete elimination of nitrogen species ($NH4^+$-N, NO_2-N, and NO_3-N), chemical oxygen demand (COD), and total phosphorus was achieved. This resulted in microalgal biomass productivity of 0.094 g/L/day [175,176].

14. Potential Applications and Important Findings

Increasing awareness of the health benefits associated with micro- and macroalgae has led to a growing demand for these organisms, as they are rich in various nutrients. This expanding market highlights the potential for microalgae in diverse industries (Figure 6) [1,145,154,168,177].

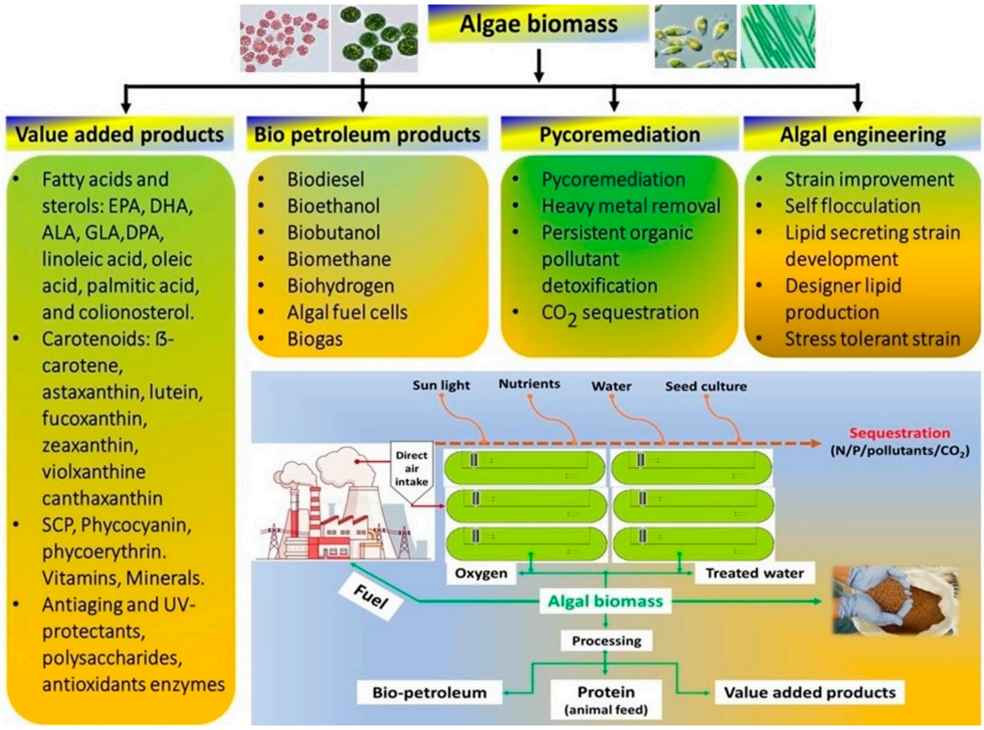


Figure 6. Potential applications and algal engineering.

a. Certain species of algae have demonstrated the ability to effectively remove heavy metals from streams, offering an environmentally advantageous solution [178]. Moreover, these algae species have a higher capacity for synthesizing carbohydrates as conserved polymers rather than lipids. This makes them promising candidates for bioethanol production, as the isolated carbohydrates can be converted into fermentable sugars [45].
b. Algal biomass possesses great versatility and is capable of being used to produce proteins, pigments, polymers, and other valuable compounds alongside bioethanol [104,179]. Additionally, algal biomass contributes to nutrient recycling in nearby waters and coastal areas while also serving as a substitute for fossil fuels, thereby mitigating climate change [180].
c. Macroalgae, as a potential feedstock for cellulosic ethanol, exhibit a high biomass yield surpassing many terrestrial crops [23].
d. The vast oceanic expanse in coastal regions, which falls within the exclusive economic zone, presents an opportunity for the cultivation of algae biomass. Utilizing seawater for algal growth shows promise in addressing water scarcity issues. Ecologically, macroalgae play a significant role in reducing atmospheric carbon dioxide levels and oxygenating water bodies [181].
e. Microalgae offer a multitude of possibilities for sustainable biofuel production, including biodiesel derived from microalgal oil [32], biohydrogen generated through photobiological processes, and methane produced via anaerobic digestion of algal biomass [182].
f. Exploring the inherent ability of plants to accumulate metals during growth provides opportunities for developing sustainable methods of nanoparticle synthesis and utilization in future bio-refinery systems [170,183].

In addition, algal-based bioenergy production is a multifaceted endeavor, encapsulating strengths, weaknesses, opportunities, and threats (SWOT) while considering life cycle assessment (LCA) across various process pathways [184]. Recent scientific articles underscore the strengths of algal bioenergy, including rapid growth rates, carbon sequestration potential, versatile biomass types, and adaptable cultivation techniques [12,43,127].

However, acknowledged weaknesses encompass cost challenges, efficient lipid extraction, potential contamination, and land–water requisites [74,176,185]. The opportunities lie in bioproduct diversification, genetic advancements, and integrated systems [32,186]. Concurrently, threats such as resource competition and ecological impacts necessitate careful navigation [26]. LCA, as highlighted by Sun et al. [187], emerges as an essential tool to evaluate the environmental footprints of distinct process pathways. For instance, the study by Clarens et al. [25] emphasizes the significance of LCA in comparing algal bioenergy with conventional feedstocks. By combining SWOT analysis and LCA, a comprehensive framework emerges, guiding the trajectory of algal-based bioenergy production toward sustainability and efficacy.

These findings highlight the immense potential of algae in various industries, from bioenergy production to environmental remediation and the creation of value-added products. Further research and development in the field of algae-based technologies will pave the way for sustainable and innovative solutions.

15. Conclusions

This article discusses the economic drawbacks of synthetic fuels and the potential benefits of using algae biofuels as alternative feedstocks to traditional biofuels. Additionally, it highlights the limitations of first- and second-generation plant-based biofuels in the food versus energy debate.

Despite high production and energy costs, microalgae are promoted as a third-generation biofuel feedstock due to their rapid growth rate, capacity to sequester greenhouse gases, and high lipid synthesis. Using microalgae for bioremediation of food wastewater can increase biomass production, lipid synthesis, and value-added product creation. Concerns about sustainability in transitioning to biofuels have raised awareness of unfavourable externalities related to resource distribution and food availability. This paper examines the pros and cons of parallel biofuels and suggests a regulatory framework to encourage the growth of third-generation algal biofuels, which offer significant positive externalities as a biofuel source.

Increased use of biomass-derived biofuels is necessary due to limited crude oil availability. Algal biofuels have shown potential in engines but require further investigation. Terrestrial feedstock utilization is complex. However, there is still a long way to go before algae-based biofuels become a viable commercial alternative to fossil fuels. The utilization of terrestrial feedstock has involved complex treatment and production processes.

In conclusion, further research, technological advancements, and supportive governmental policies are crucial for the successful development and commercialization of microalgal biofuels. By addressing the existing limitations of traditional biofuels and exploring the promising attributes of microalgae, we have identified a significant opportunity for sustainable energy production.

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