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Review

Biofuel Production Using Cultivated Algae: Technologies, Economics, and Its Environmental Impacts

Motasem Y. D. Alazaiza [1,*], Ahmed Albahnasawi [2], Tahra Al Maskari [1], Mohammed Shadi S. Abujazar [3], Mohammed J. K. Bashir [4], Dia Eddin Nassani [5] and Salem S. Abu Amr [6]

1 Department of Civil and Environmental Engineering, College of Engineering, A'Sharqiyah University, Ibra 400, Oman
2 Department of Environmental Engineering, Gebze Technical University, Kocaeli 41400, Turkey
3 Al-Aqsa Community Intermediate College, Al-Aqsa University, Gaza 4051, Palestine
4 Department of Environmental Engineering, Faculty of Engineering and Green Technology (FEGT), Universiti Tunku Abdul Rahman, Kampar 31900, Malaysia
5 Department of Civil Engineering, Hasan Kalyoncu University, Gaziantep 27500, Turkey
6 International College of Engineering and Management, Seeb 2511, Oman
* Correspondence: my.azaiza@gmail.com

Abstract: The process of looking for alternative energy sources is driven by the increasing demand for energy and environmental contamination caused by using fossil fuels. Recent investigations reported the efficiency of microalgae for biofuel production due to its low cost of production, high speed of growth, and ability to grow in harsh environments. In addition, many microalgae are photosynthetic, consuming CO_2 and solar light to grow in biomass and providing a promising bioenergy source. This review presents the recent advances in the application of microalgae for biofuel production. In addition, cultivation and harvesting systems and environmental factors that affect microalgae cultivation for biofuel production have also been discussed. Moreover, lipid extraction and conversion technologies to biofuel are presented. The mixotrophic cultivation strategy is promising as it combines the advantages of heterotrophy and autotrophy. Green harvesting methods such as using bio-coagulants and flocculants are promising technologies to reduce the cost of microalgal biomass production. In the future, more investigations into co-cultivation systems, new green harvesting methods, high lipids extraction methods, and the optimization of lipid extraction and converting processes should be implemented to increase the sustainability of microalgae application for biofuel production.

Keywords: microalgae; biofuel; cultivation and harvesting; techno economic assessment; lipid extraction

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1. Introduction

Energy is an essential infrastructure for economic growth and social advancement due to the rapid expansion of the world's population and the development of industrialization. Using fossil fuels as a primary energy source in the last century has resulted in a slew of negative consequences, including climate change, ecological degradation, and biotic health issues [1,2]. Fossil fuel combustion releases CO, CO_2, SOx, and NOx gases into the atmosphere, which exacerbates greenhouse effects and causes global warming, endangering the balance of the world's ecosystems. If emissions are not limited, the present CO_2 level of 394.5 ppmv (parts per million volume) is expected to increase to 500 ppmv by 2050 [3]. Therefore, in order to address the energy crisis, it is crucial to rely on a sustainable and promising energy source alternative [1]. There is a high demand for alternative fuels because of rising environmental issues and the depletion of global fossil fuels. Given that they are environmentally friendly, clean, sustainable, and renewable, biofuels are currently thought to be among the greatest alternatives to fossil fuel [4]. One of the main advantages of biofuel is that it can be used with some or no modifications in any

compression-ignition engine, especially in heavy-duty automobiles and marine engines, either alone or in combination with petroleum diesel [5,6].

Microalgae are currently receiving a lot of attention as a potential alternative energy source [7]. Due to their better biomass output, adaptability to grow in a variety of environmental medias, and higher oil or lipid content, scientists, researchers, and fuel companies are taking microalgae into consideration as a viable biofuel feedstock [8,9]. Microalgae are referred to as a third-generation biofuel feedstock that doesn't compete with food and/or land crops. The cultivation can be done on undeveloped land, open land, or closed land [10]. The raw material for first-generation biofuels is edible oil, which includes vegetable, palm, soybean, corn, wheat, moringa, and oil from other plants [11]. According to previous reports, first-generation biofuel has negative effects on deforestation, global food markets, water availability, and food security [12]. As a result, there is a lot of debate about these types of biofuels [13]. Additionally, second-generation biofuels made from non-edible oils (such those from *Jatropha curcas*, *Simarouba glauca*, *Pongamia pinnata*, and others), forest waste, and lignocellulose biomass require the coverage of large areas of land that could be used for growing food [14,15]. The development of second-generation biofuels currently faces the lack of technology that is efficient for turning waste goods into a source for creating biofuel. Based on the aforementioned issues, microalgal biofuel is typically a viable alternative energy source to replace or supplement fossil fuels [16]. Fourth generation biofuels, a new type of biofuel, can be created by genetically altering microalgae. Microalgae-derived fuel provides advantages, such as biodegradability, being ecologically beneficial, and high energy generation [17]. Compared to other feedstocks, microalgae produce a significantly higher volume of oil and can be harvested at any time of year, which makes it very handy [18]. Both open and closed systems can support microalgal growth. The supply of various nutrients and CO_2, which are necessary for microalgal development, can be obtained from wastewater streams and the combustion of fossil fuels, respectively [19]. Moreover, microalgae transform CO_2 into chemical and fuel products, resulting in less air pollution, which is considered a profitable technique to reduce greenhouse gas emissions while still maximizing financial gains [20]. A few examples of microalgae that contain significant levels of lipids and hydrocarbons include *Botryococcus braunii*, *Nannochloropsis* sp., *Dunaliella primolecta*, *Crypthecodinium cohnii*, and *Chlorella* sp. [21]. Oil content in microalgae species can reach 80% while normal ranges are between 20% and 50% [22]. For example, the microalga *Chlorella* contains up to 50% lipids, and *Botryococcus braunii* have the highest oil content, which reaches up to 80%. In addition to creating biofuel, microalgae also create a variety of bioactive compounds that have a wide range of applications in medicine, nutraceuticals, and chemicals [23,24].

The main objective of this study was to provide readers with an overview of microalgae applications for biofuel production. This review covers the main topics regarding microalgae cultivation and harvesting, lipid extraction and conversion, and a techno-economic analysis for sustainable biofuel production. The first section discusses and presents the microalgae cultivation systems, microalgae growth influenced factors, harvesting methods, and drying process techniques. The second section presents the lipid extraction approaches and the lipid to biodiesel conversion methods. Finally, the review discusses the techno-economic analysis and environmental impacts of microalgae cultivation.

2. Cultivation Systems and Harvesting Methods

2.1. Microalgal Cultivation Modes

The composition and growth dynamics of microalgae are significantly affected by the mode of growth. Based on carbon supply and energy, heterotrophic, photoautotrophic, mixotrophic, and photoheterotrophic are the main growth modes of microalgae. In this section, a brief review of these cultivation modes is presented. Most microalgae are photosynthetic, where CO_2 is the source of carbon. Microalgae use solar electromagnetic energy as an energy source to convert CO_2 to organic matter. This mechanism is the main advantage of the photoautotrophic cultivation mode over the other modes, which mitigate

the global CO_2 problem. The microalgae in phototrophic cultivation use the energy from light to produce organic substances for food [25,26]. Depending on the species used, the lipid content of microalgae during phototrophic cultivation might range from 5 to 60% [27]. However, phototrophic cultivation depends on sufficient CO_2 supplies and light intensity. Therefore, increasing CO_2 concentration by directly adding CO_2 or using industrial fuel gases rich with CO_2 could increase the productivity of lipids [28].

In contrast to photoautotrophic cultivation, heterotrophic microalgae cultivation is conducted in a dark environment using organic compounds as sources of energy and carbon [29]. While the heterotrophic cultivation mode has advantages, such as a short period of cultivation and high productivity, obtaining a high volume of value-added products and the presence of organic matter, which is affected by the microbial community, is the main shortcoming of this cultivation mode [30]. Many algae strains, such as *Chlorella protothecoides*, *Chlorella vulgaris*, *Crypthecdinium cohnii*, and *Schizochtrium limacinum*, can be cultivated without light. Moreover, some algae species cannot cultivate in the phototrophic mode and need organic carbon in the absence of light. Thus, heterotrophic cultivation can avoid the limitation of light and result in high yield biomass production. In addition, the harvesting cost may decrease with the high cell density [31]. Overall, heterotrophic culture may increase cell growth rate, lipid accumulation, and biomass compared to photoautotrophic culture [32].

In mixotrophic cultivation, CO_2 and organic carbon are consumed at the same time by microalgae where photoautotrophic and heterotrophic metabolism simultaneously takes place. Mixotrophic is a two-stage mode where heterotrophic cultivation occurs first due to the high content of organic carbon. Following the consumption of organic carbon to a certain level, the photoautotrophic mode starts; therefore, the algae consume CO_2 photoautotrophically, indicating the start of the second stage. The limitations of the autotrophic and heterotrophic cultivation modes can be handled in a mixotrophic mode. In mixotrophic cultivation, the overall CO_2 emission is lower than in heterotrophic cultivation as microalgae swallow inorganic carbon through photosynthesis and produce oxygen. Generally, mixotrophic cultivation differs from autotrophic and heterotrophic cultivation in terms of the properties of photosynthesis [33]. In contrast to autotrophic cultivation, mixotrophic cultivation has a higher respiration rate. Furthermore, under mixotrophic conditions, the amount of photosynthetic pigment and the activity of the photosystem vary. However, the type of microalgae and the organic carbon supply affect the photosynthetic pigments [34].

Under photoheterotrophic conditions, microalgae need light to grow and consume organic matter as a source of carbon. The main difference between photoheterotrophic systems and mixotrophic cultivation is that in photoheterotrophic systems, light is used for energy, nitrogen fixation, and organic matter that serves as a carbon source without producing carbon dioxide [35]. Although organic carbon and light are necessary for photoheterotrophic cultivation, it is rarely used to produce microalgal biomass for the creation of goods with added value [30].

2.2. Parameters Influencing the Growth of Algae

Physical factors and medium characteristics affect the lipid content of microalgae during its life cycle [36]. Algae energized by sunlight create carbohydrates, lipids, and proteins during photosynthesis, which is dependent on a number of variables, such as light intensity, temperature, CO_2 levels, and pH levels [37].

2.2.1. Effect of Temperature

Temperature significantly affects the lipid content of microalgae, and as a result, temperature is considered an important physical element [38]. Temperature influences a strain's physiological functions by speeding up or slowing down metabolic reactions within cellular compartments [39]. Some microalgae strains are not affected by the change in temperature, whereas some microalgae are affected by temperature changes. For instance,

Chlorella sorokiniana did not exhibit a significant change in lipid content while *N. salina (an eustigmatophyte*) and *Ochromonas danica (a chrysophytan)* showed a similar rise in lipid content with a rising cultivation temperature [40]. Kalacheva et al. [41] investigated the temperature effect on the lipid content of the Botryococcus *braunii* strain. The results showed variations in lipid content at different temperatures (32, 25, 18) °C. Compared to 22% at 25 °C, intracellular lipid concentration was reduced to 5% at a temperature of 32 °C, extracellular lipid synthesis was unaffected, and polysaccharides increased. Noticeably, the temperature at which *Chlorella vulgaris* and *Nannochloropsis oculata* grow best is 25 °C. A temperature change from 20 to 25 °C resulted in a two-fold increase in *N. oculata*'s lipid content from 7.90% to 14.92% while a temperature change from 25 to 30 °C resulted in a decrease in *C. vulgaris*' lipid composition (14.71% to 5.90%) [42]. Fakhry and El Maghraby [40] investigated how a changed temperature affected TAG (triglyceride) and the lipid content of *Nannochloropsis salina*. The authors stated that when the temperature was raised from 15 to 35 °C, the content of fat and triglycerides rose. Microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* were cultured at various temperatures, namely 15, 20, 25, 30, and 35 °C, to examine the impact of temperature on the fatty acid content. Neutral lipid and polyunsaturated fatty acid (PUFA) levels decreased at high temperatures (supra-optimal), but monoun-saturated fatty acids (MUFA) levels increased [43].

2.2.2. Effect of Light Intensity

For microalgal growth, spectral range and photoperiod of light are thought to be an essential environmental component [43]. As microalgae develop, their profile, lipid yield, and photosynthetic activity alter noticeably at different light intensities and wavelengths. High light intensity increased the concentration of triacylglycerol (TAG) but decreased the composition of polar phospholipids in the filamentous green alga Cladophora sp., according to Liu et al. [44]. In this regard, it was discovered that increasing the light intensity from 700 to 1500 μmol/m^2/s caused *D. viridis* to produce 63% less biomass [45], but the amount of extracellular polysaccharide in the organism can be increased as high photon flux density exposure is applied [46]. *D. virdis* was shown to have a higher total lipid content when dark incubated (without light), while simultaneously having a lower composition of triglycerides and free fatty acids [47]. The total lipid of *D. salina*, *N. oculate*, and *Isochrysis galbana* increases when the photon flux is increased up to 150 μmol/m^2/s [48]. On the contrary, *Chlorella* sp. and *Nannochloropsis* sp., two marine microalgae that flourished in environments with high 10,000 lx photon densities, showed low total lipid yield [49]. Durairaj et al. [50] looked into how different light colors and intensities affected the lipid yield of the axenic microalga *Chlorella* sp. At 2700 lx, the maximum lipid content was 19.4%, which was higher than in other cultures exposed to different light intensities. When compared to yellow and white light-produced cultures (the controls), cultures developed under red light exhibited a higher lipid content; conversely, in the same system, the produced lipid was lower by 7.2% when green light was used. Despite the fact that light is what drives both photosynthetic and photo acclimation processes, different strains and species react differently to photo stress. For instance, *Chlorella* sp. cultivated in a light-illuminated environment for 24 h produced high levels of biomass (0.54 g/L) and lipids (0.0791 g/L).

2.2.3. Effect of CO_2

Bio-fixation of carbon dioxide using microalgae reduces CO_2 emissions from both atmospheric sources and more concentrated ones like industrial flue gases and power plants. Whereas the former is more promising because a higher CO_2 level (up to 20%), which improves carbon uptake, the latter is simple to implement but inefficient due to a low carbon concentration.

Green algae may cultivate easily in environments with higher CO_2 concentrations as microalgae, such as *Chlorella*, have a high photosynthetic efficiency when CO_2 is being sequestered [51]. As a result, the production of microalgae from point-source genuine flue gases appears to be quite intriguing. However, dangerous substances like NOx and SOx

may be present in flue gases, which warrants further study. The lipid (fatty acid) content and the growth rate are the two specific properties that may be impacted by the CO_2 level. In this regard, numerous studies have looked into how CO_2 affects the development of lipids and the content of fatty acids for numerous microalgal culture. Yoo et al. [52] explored how three distinct strains responded to CO_2 concentrations ranging from 0% to 10%. The species were *Scenedesmus* sp., *Chlorella vulgaris*, and *Botryococcus braunii*. The author observed that all three species can grow up to the highest CO_2 levels (10%). They also found that after a two week cultivation period, the three strains produced varying amounts of biomass and total lipid output. *Botryococcus braunii* has specifically shown a biomass productivity (26.55 mg/L/d) and a total lipid production (5.51 mg/L/d). Similarly, *Chlorella vulgaris* biomass productivity was 104.76 mg/L/d, and the total lipid production was 6.91 mg/L/d; for *Scenedesmus* sp., biomass productivity was 217.50 mg/L/d, and the total lipid production was 20.65 mg/L/d. Two of the aforementioned strains were evaluated in the same investigation in an actual flue gas atmosphere with a carbon dioxide content of 5%. The species biomass productivity was shown to be improved in these circumstances, but *Scenedesmus splipid.*'s productivity gained the most attention since it increased by 3.7 times to 21 mg/L/d or 24% w. In another study, Ge et al. [53] investigated the effect of CO_2 (up to 20%) on *Botryococcus braunii*. Their findings showed that when CO_2 concentration increased from 2% to 20%, the hydrocarbon yield increased from 16.43 to 24.45%, whereas the total lipids increased from 10.41 to 12.71%. Furthermore, the length of time that CO_2 is supplied to the culture is also thought to be an important issue that influences the fatty acids contents. More specifically, taking *Dunaliella salina* as an example, the increase in CO_2 from 2% to 10% for one day results in a small increase in the total acids, but 7 dyes of CO_2 at the same level resulted in a 270% increase in fatty acids contents [54]. In general, the microalgae growth is promoted by the increase of CO_2 concentration in culture media. Nevertheless, this is linked to the microgel species and constrains. In fact, as demonstrated by Chiu et al. [55], some microalgae constrains such as (*Chlorella* sp.) showed declining growth rate as long as the CO_2 concentration rises. To sum up, once the carbon dioxide tolerance threshold has been determined, it is a good idea to increase the CO_2 concentration in the culture media to the amount necessary for the strain's maximal productivity. From the perspective of the environment, numerous studies used untreated and desulphated flue gas as an inorganic CO_2 source. Interestingly, no discernible variation in the biomass content of *Phaeodactylum tricornutum* and *Nannochloropsis salina* was observed. Additionally, as NOx is absorbed by cells as a source of nitrogen, the presence of trace amounts of mercury, vanadium, and nickel in the flue gas may impair the growth rate [56].

2.2.4. Effect of pH

The pH controls the distribution and availability of the critical nutrients and inorganic carbon sources; thus, it is an important parameter for microalgal cultivation [57]. Since pH influences the availability of inorganic nutrients through metal speciation and carbon, the photosynthetic process, the rate of lipid synthesis, and the activities of enzymes within the cell are also affected by pH [58]. The pH has an impact on microalgal metabolism as well, and each strain or species of algae has a different optimal pH range [57]. Algae respond to changes in the pH of the growing medium by changing their biomass and lipid output. At the time of inoculation, the pH of the algal growth medium is initially kept neutral. Because the pH of the medium controls the growth of carbonaceous species, the pH of the algal suspension rises linearly throughout the day because of the use of inorganic carbon. Nevertheless, a high pH in the media reduces the affordability of CO_2, which inhibits the productivity of microalgae [59].

The impact of pH on the biomass and lipid content of algae has been determined by several researchers. The ideal pH was discovered to be 6.5 for *Chlorella protothecoides* [60]. To investigate the effect of various pH levels and inorganic carbon sources, the biomass and lipid productivities of *Tetraselmis suecica* CS-187 and *Chlorella* sp. were examined. *T. suecica* had high biomass and lipid productivities of 320 mg/L/d and 92 mg/L/d at

pH 7.5, whereas *Chlorella* sp. had 407 mg/L/d and 99 mg/L/d at pH 7.0, respectively [56]. Additionally, the effects of various pH ranges (6–10) on the lipid accumulation and biomass production of *Nannochloropsis salina* were assessed. The results showed that *N. salina* exhibited its highest productivity at pH 8.0 and 9.0 and that the lipid content of the unbuffered system was 21.8% fatty acid methyl esters (FAME) (on a mass basis). Furthermore, the study came to the conclusion that at such pH levels, lipid production was at its peak, and microbial invasion was at its lowest [61]. The environmental variables affecting the growth of microalgae biomass production. The environmental variables affecting growth of microalgae biomass production (optimal condition for biomass) are summarized in (Supplementary Table S1).

2.3. Types of Microalgal Biomass Production Systems (Reactor)

Open pond and closed system photobioreactors (PBR) are the two types of microalgae cultivation systems (Figure 1). For the production of biomass and wastewater treatment, open pond cultivation methods are used. Open ponds are simple to set up, maintain, and manage [62,63]. PBR are closed vessels with a mother culture, artificial light source, medium, and rotator. They have advantages of having zero gas exchange and being contaminant-free. Due to its ability to regulate environmental factors like light, pH, and CO_2, and temperature, PBR systems have benefits over open pond systems. PBR systems have advantages over open pond cultivation. PBR systems have higher operational and maintenance costs than open pond cultivation; however, their biomass concentration grows at a rate of 20 to 100 g/day/m^2, which is faster than the cultivation rate of an open pond, which is 10 to 25 g/day/m^2 [64].

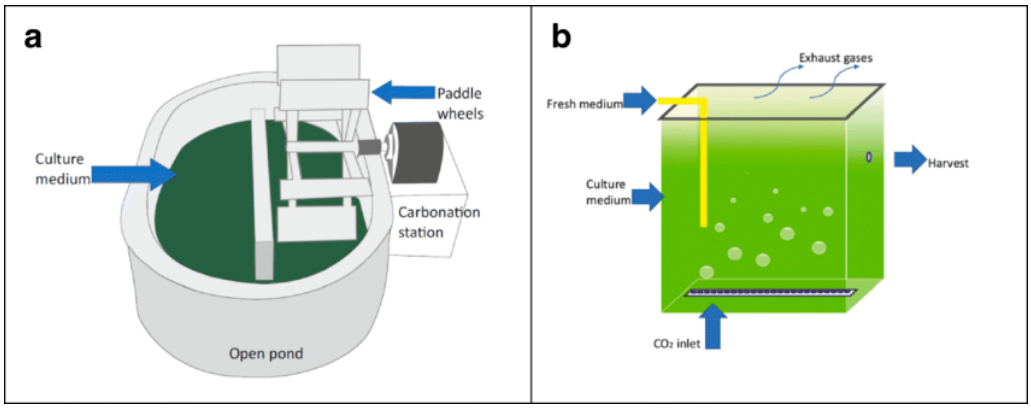

Figure 1. (**a**) Open raceway pond, (**b**) photobioreactor. Reprinted with the permission from [65]. Copyright 2022 Springer Nature.

2.3.1. Open Pond Cultivation Method

The three types of open pond cultivation are raceway ponds, circular, and stirred. The key considerations for open pond design are gas/liquid mass transfer, mix efficiency and residence time, and light penetration. To mix the open ponds, both a paddle wheel-driven motor and a manual paddle are used. Unstirred ponds can be easily built with a depth of lower than half a meter and protected in plastic films, but they grow slowly because of environmental conditions [66]. Raceway ponds are built in a variety of designs with parallel rectangular channels that allow water to flow through a paddle wheel drive coupled to a motor. Raceway ponds have dimensions of 10 to 100 meters in length, 1 to 10 meters in width, and 0.25 to 0.30 meters in depth [66,67]. Due to their ability to remove nutrients from wastewater, open raceway ponds are affordable and useful for producing microalgae. Currently, greenhouse gases are used in the construction of open ponds to reduce debris, rainfall, water evaporation, and pollution [68]. For greater biomass production, large ponds with dimensions of 30 × 10 m are built and protected with plastic bags with an 80 cm wall slope [66].

2.3.2. Photobioreactors (PBR)

Closed-system cultivation entails the growth of isolated algae in a closed reactor with all PBR parameters, such as CO_2, light, circulation systems, and medium [69,70]. The selection of a reactor is the primary element for biomass growth rate in closed bioreactors, which produce five times as much biomass compared to open systems. Reactors can be classified as flat plate, column, membrane, and tubular PBR. For the manufacturing of biofuel, semi-hollow spheres are also employed [32]. However, microalgae were found to produce between 20,000 and 60,000 oils per acre per year while using less arable land [68]. PBR have been modified to produce more biomass. For example, flat plate photobioreactor is created by polycarbonate, plexiglass, and transparent glass. High cell proliferation with a density less than 80 g/l and a high surface-to-volume ratio are some of its advantages [71,72]. Compared to a standard PBR, the flat plate photobioreactor has a larger lighted area, which positively affects biomass production. For example, Khan et al. [73] found that biomass production increased by 220% when the lighted area increased by 410%. In comparison to typical PBR, which produce 0.264 kg/m^3, flat plate photobioreactors that emit red light from both sides enhance the production of biomass to reach 0.326 kg/m^3 [74]. The polymethyl methacrylate (PMMA) tubes inserted into flat plate photobioreactors boosted the efficiency of photosynthesis by 12.52. Moreover, the degree of medium mixing is increased, and the rate of algal growth is accelerated by flat plate PBR attached to mixers [75]. For example, the productivity of biomass is increased by 1.88 times in flat plate PBR compared to reactors without baffles [76]. Another type of PBR is the tubular reactor, which is split into horizontal and vertical bioreactors [66,77].

2.3.3. Hybrid Cultivation System

Contamination and high costs are disadvantages of both open-pond and PBR cultivation methods. To prevent pollution and enhance cell development, it is advised to combine PBR with an open pond. A hybrid reactor can be used to develop a particular algae species, which can then be transported to an open pond where the inoculum should be high enough to outcompete contaminants [78].

2.4. Microalgal Cultivation Strategies

The efficacy of producing microalgae-derived biofuels is dependent on the culture strategy selected. Batch, semicontinuous, and continuous culture techniques are the three primary categories. All of the techniques employed in microalgal cultivation have pros and cons in terms of lipid productivity and economic viability, which are discussed in this section.

2.4.1. Batch Cultivation

In this method, the biomass is cultivated for a set amount of time in a nutrient culture under specific operation conditions. As a result of the increased development of cultured algae, the nutrient content steadily drops. The biomass of *Micractinium inermum* NLP-F014 increased every day under batch cultivation until day 10, when it reached 5.10 g/L, at which point the growth marginally slowed down as a result of nutrient consumption [79]. In this method, a variety of variables, including nutrient concentrations, pH, temperature, and dissolved oxygen, directly affect microalgae growth. Of all the cultivation techniques, the batch approach is the most simple, adaptable, and reliable. On the other hand, the main drawbacks of this approach are the challenges associated with maintaining strains in the continuous exponential phase, the restriction to a monoculture cultivation cycle, the rapid nutrient consumption, and the unequal exposure to irradiance as a result of the cell self-shading effect, which ultimately results in decreased biomass yield [80].

2.4.2. Continuous Cultivation

The primary characteristic of continuous cultivation is that biomass develops under steady-state circumstances, meaning that cell division happens steadily in a controlled

environment [81]. The continuous culture approach initially resembles batch cultivation in that both are given the nutrients needed at the start of process. Nevertheless, in continuous mode, new nutrients are continuously introduced to maintain the algae in a log phase, allowing cells to expand at an unexpected rate. As a result, the growth rate rises dramatically [82]. By employing 10%, 5%, 7.5%, and 7.6% of landfill leachate, respectively, as a nutrition supply while cultivating *Chlorella minutissima* continuously, Tagliaferro et al. [83] obtained the highest carbohydrate (33.40 mg/L/d), lipid (96.60 mg/L/d), protein (47 mg/L/d), and biomass productivity (232 mg/L/d). The continuous cultivation method has a higher biomass density, controlled degree of automation, and the ability to manage nutrients and physical conditions to produce a continuous supply of high-quality cells. The difficulty in maintenance, complexity, and high equipment costs are the main challenges that prevent it from being widely used.

2.4.3. Semi-Continuous Cultivation

This technique involves frequently removing some full-grown algal cultures and using the remainder as the initial inoculum for a new batch while replenishing all the nutrients needed to establish the new culture. Compared to batch culture, this method can generate a much higher number of repeated cycles and aid in maintaining microalgae cultures in the exponential phase for longer, resulting in increased biomass production. For instance, Fernández-Linares et al. [84] reported that the highest cellular biomass, carbohydrates, lipids, and proteins, were obtained by cultivating a microalgal consortium, using piggery wastewater as a source of nutrients, for 112 days. However, in other instances, it is impossible to carry on the cultivations because of the rising levels of contaminants, competitors, and metabolic waste. Because of its high biomass production and less complicated harvesting process compared to batch cultivation, the semi-continuous approach is suggested by scientists as a more practical approach to commercial-scale growth [85]. He et al. [86] investigated the microalgae cultivation and lipid production costs of *Chlorella* sp. L1 and *Monoraphidium dybowskii* Y2 species. The results showed that under semi-continuous mode, *Chlorella* sp. and *M. dybowskii* produced the highest growth rate of 19.35 and 18.23 g/m^2/d, respectively, as opposed to 17.03 and 9.94 g/m^2/d, respectively, under batch mode. Additionally, the lipid production costs of *Chlorella* sp. and *M. dybowskii* in the semi-continuous mode were 14.18 and 13.31 dollars per gallon, respectively, as opposed to 21.0 and 16.54 dollars per gallon in the batch method.

An LCA calculation of *C. vulgaris* microalgae production in outdoor raceway ponds using semi-continuous and batch techniques was carried out by Yadav et al. [87]. According to their findings, semi-continuous culture produced the highest levels of biomass density and production, which were 0.42 g/L and 11.488 g/m^2/d, respectively, as opposed to the batch culture's 0.30 g/L and 3.27 g/m^2/d values. Additionally, the semi-continuous method was capable of fixing 102.66 mg/L/d of CO_2, demonstrating the benefits of adopting a semi-continuous raceway pond microalgal culture next to thermal power plants to reduce the carbon footprint and use the waste CO_2. In comparison with batch culture, semi-continuous and continuous cultivation typically yield a faster productivity with less wastewater formation (the water is used longer). Nevertheless, there are several concerns with these techniques that must be addressed, including the uncertain stability for long-term operation and the high energy requirement [80]. As a result, creating sophisticated growing techniques is necessary for commercial microalgae uses. The comparison of different microalgae cultivation systems and modes can be found in (Supplementary Materials Table S2).

2.5. Microalgae Harvesting Techniques

According to many studies, the cost of harvesting makes up at least 20% of the entire cost of producing microalgal biomass [88,89]. It is crucial to use a method that is both effective and affordable for harvesting microalgae from a liquid growth medium. Despite the advantages of cost-effective algae production, lowering the cost of harvesting algae is still a significant challenge in the downstream process of using microalgae to

treat wastewater and produce biofuels and other high-value bioproducts [90]. Due to the small size of algae and their close density to water, industrial-scale processing is still greatly hampered by the difficulty of removing the algae from large quantities of liquid medium. Choosing proper harvesting method is highly dependent on algal strains and medium properties.

2.5.1. Centrifugation

Centrifugation is a typical technique for separating algal cells from their culture media. The benefits of using centrifugation include continuous processing, high harvesting efficiency, and the convenience of use. The parameters of sedimentation, the depth of sedimentation, and the length of the algae's stay all affect its efficacy. On the other hand, when the rate of harvesting was high, the energy consumption was similarly significant based on the economics of centrifuging microalgae [91]. However, by increasing the algae flow rate, the overall energy usage can be decreased. When just 28.5% of the biomass of *Nannochloris* sp. was harvested with centrifugation at a low rate of 18 L/min, it was shown that energy consumption would be reduced by 82% [92]. Nevertheless, while centrifugation is an effective method for microalgae harvesting, many disadvantages of using such technology include the disruption of microalgae cells due to strong gravity and shear stresses [93] and its possible labor- and time-intensiveness. It can also require expensive equipment that can be replaced by alternative technology [94].

2.5.2. Sedimentation

Sedimentation applies gravity force to collect microalgae. The density of algal cells has a major impact on sedimentation efficiency, where microalgae with large cell densities are best suited for rapid sedimentation. However, other influencing elements, such as dissolved oxygen, may impact the effectiveness of sedimentation; when the concentration of oxygen produced by photosynthesis is above the saturation of dissolved oxygen, it may cause bubbles that prevent the sedimentation of the algal cells [95]. The most popular technique in large-scale facilities is sedimentation without the addition of chemicals; however, due to their propensity to float naturally in order to catch sunlight, many microalgal species are extremely challenging to separate by gravity settling without chemical aids [1].

2.5.3. Flotation

A high number of tiny bubbles are used in the flotation process as a carrier to strike suspended algae cells. This forms a foam of algal particles on the surface of microalgae cells by reducing their density, making them float to the liquid surface. The efficacy of this harvesting technique and the ensuing separation are dependent on system variables, such as dose, algal concentration, agitation speed, pH, and flotation time. According to studies, some microalgae species, such *Chlorella* sp. cells, can be extracted by froth flotation at a rate of up to 98.4%. [96]. The harvesting period is quite brief, and the yield is often rather high with this flotation technique. However, the primary obstacle to using this approach is still how to manufacture microbubbles with great efficiency and little energy usage [97].

2.5.4. Flocculation

Flocculation is a method of gathering microalgae by neutralizing, bridging, or netting, which collects the dispersed charged cells into larger, denser clumps to accomplish solid–liquid separation; this technique for extracting microalgae cells uses chemical, biological, and electro-flocculation methods. Although the three approaches have certain unique properties, flocculation is increasingly being explored as a potential low-cost harvesting technique [98]. Flocculation is simply one step in the microalgal harvesting process; to acquire the algal biomass, another mechanical step like dewatering is then needed [99]. A comparison of different microalgal harvesting techniques is shown in Supplementary Table S3.

2.6. Drying Processes

Due to the high energy needed and high cost to dry produced algal biomass, the drying phase is one of the biggest issues the biofuels business faces. Biodiesel yield is significantly impacted by the high-water content of biomass, which has an impact on lipid extraction. Water interferes with the interaction between lipids and solvents during the extraction of lipids, lowering the extracted lipids' yield [100]. The drying process can be divided into five categories depending on the source of the dryer: (1) solar drying, (2) air drying (including hot air drying, tray drying, heat pump drying, and fluid bed drying), (3) atomization drying (including spray drying and spouted bed drying), (4) radiation drying (including microwave oven and infrared drying), and (5) vacuum drying (including vacuum cast tape drying, freeze-drying, and vacuum drying). Many methods, including oven-drying, sun-drying, spray-drying, and freeze-drying, are employed in the preparation of biomass to produce biodiesel. The type of extraction process that will be utilized on production scales and the characteristics of the extracted product will generally determine the most appropriate drying procedure. Sun energy can be used directly by using direct solar radiation or indirectly by drying wet microalgae biomass with a solar water heating system. Although direct sunlight is effective at drying wet biomass, its instability and lack of control could lead to rapid overheating that would destroy essential biomass metabolites. The use of an indirect solar water heating system could solve these issues, but it would be more expensive [101]. In general, solar drying is affordable, efficient, and energy efficient. The disadvantages of this method include a significant loss of dried biomass, the requirement for a sizable plan area, and unpleasant odors brought on by microorganisms that become active by extending the drying process. In addition to the fluctuation of sunlight throughout the day, sunlight has temporary availability throughout the year. In addition, it requires the use of an extra heat source to guarantee the completion of the drying process, which adds to the costs and energy used.

The type and quantity of recovered fatty acids depend on the temperature used to get dry biomass. For example, the amounts of saturated and mono-unsaturated fatty acids increased as the drying temperature goes up in *Scenedesmus* sp., whereas the triglyceride content significantly decreased [102]. Since oven drying is more regulated and quicker than solar drying, it is particularly useful for reaching an acceptable level of dried biomass. However, oven drying is more energy-intensive and has greater maintenance and operation expenses than solar drying [103]. Another method that is extensively implemented in the food companies and for drying microalgae is freeze-drying, commonly known as lyophilization [104]. It has a lower risk of cell damage and preserves all the cell's components. It is also preferred for drying expensive goods, particularly those that are particularly susceptible to oxygen exposure and high temperatures. The lipid production of biomass may be increased when it is dried using a freeze-drying process, according to a report in the same vein [105]. However, the main challenges with this approach are its high operation and maintenance costs in addition to the lengthy time needed to finish the drying phase.

3. Biofuel Production

3.1. Lipid Extraction

Many methods have been investigated to extract lipids from microalgae cells (Figure 2). This section discusses and presents these methods.

3.1.1. Soxhlet Extraction Method

The gathered algae are dried and powdered into a fine powder for conventional Soxhlet extraction, which is then inserted into a cellulose thimble and placed inside the reflex chamber. This is placed on top of a flask with three necks that contain n-hexane [106]. When the solvent is boiling, it vaporizes and escapes from the flask. Hexane is condensed above the extraction chamber, dropped into the thimble, and used to wash the powder. The solvent oil flows back into the three-neck flask. A Clevenger apparatus is used to separate oil from hexane [107]. The selection of the solvent is a crucial factor in

achieving the best lipid extraction. Hexane, chloroform, diethyl ether, benzene, methanol, acetone, ethanol, ethyl acetate, and alcohol are a few examples of polar solvents that can be used for extraction [108]. Even though N-dimethylcyclohexylamine is utilized to freeze dry biomass cells, these are substantial energy users for distillation. Chemical techniques extract lipids using a variety of solvents in varying volume-to-volume (v/v) ratios, including acetone/dichloromethane, hexane/isopropanol, chloroform/methanol, dichloroethane/ethanol, and dichloroethane/methanol. Each species' cells are different in terms of permeability (ability to break the cell wall) and thickness. Therefore, picking the right solvent is crucial to improving the lipid extraction productivity. *Botryococcus braunii*, a microalga, secretes long chain lipids directly into the growth medium. Long chain hydrocarbons are without a doubt present in the retrieved lipids. As a result, the advantage of this strategy is the low-cost and straightforward conversion of long chain hydrocarbons into biodiesel. Nevertheless, because of its extremely slow specific growth rate, *Botryococcus braunii* cannot be converted into biodiesel [109]. Through the selective extraction approach, wet algal paste can be converted directly to jet fuel by utilizing hexane, ethanol, and Pt/Meso-ZSM-5 as catalyst [110].

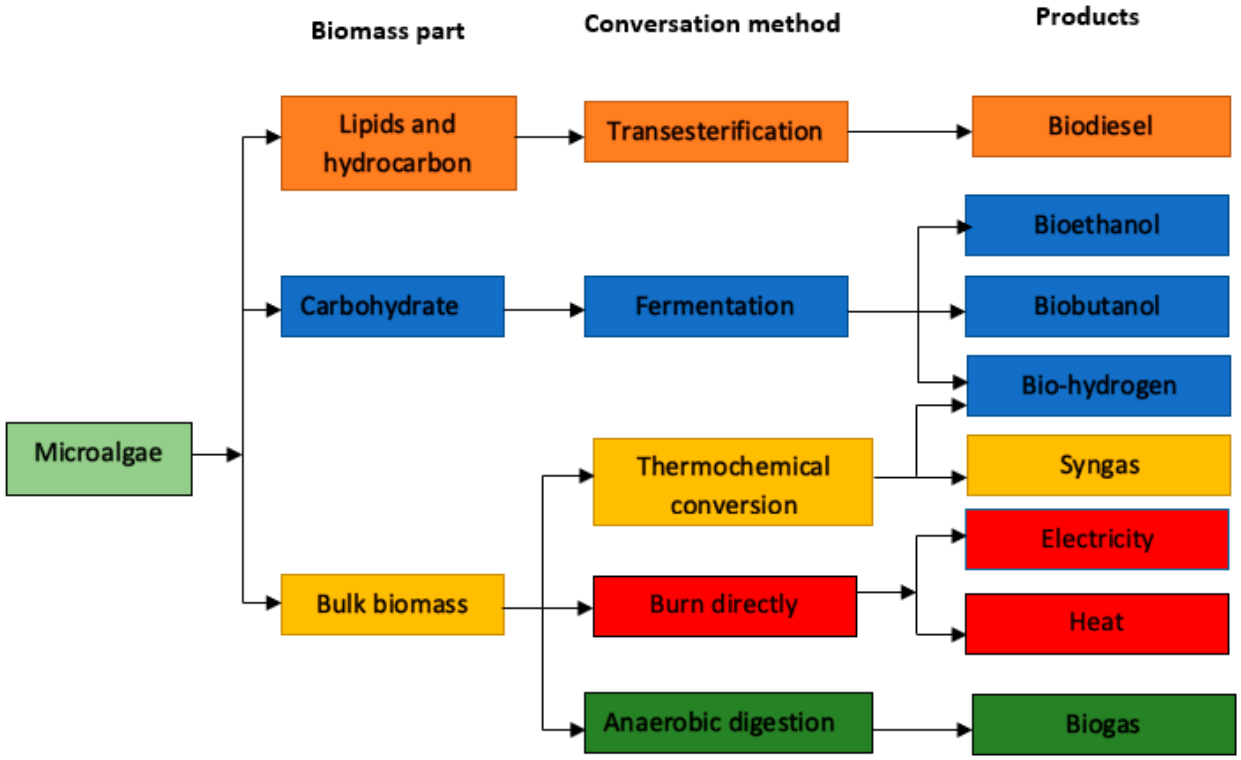

Figure 2. Different methods for biofuels/bioenergy production from microalgae.

3.1.2. Microwave Irradiation Extraction

Microwave radiation can rupture macroalga cells, resulting in localized hotspots known as polar molecules that release lipids. The extraction efficiency of this approach is higher than the Bligh and dryer method despite the fact that it requires a lot of energy [111]. To determine the best parametric conditions for oil extraction, the impact of microwave irradiation timing regarding biomass concentration and irradiation power was investigated. Increased cell disruption is made possible by the longer pretreatment period and the localized heating in the suspension. The amount of biomass suspension that underwent pretreatment rose as microwave power was increased from 635 to 1021 W. Additionally, a larger percentage of water in the biomass indicated a high rate of molecular collision and the breakdown of the microalgal cell wall [112].

3.1.3. Lipid Extraction Using Ionic Liquids and Methanol

Lipids are extracted using ionic lipids, such as $MeSO_4$ and CF_3SO_3 (trifluoro methane sulfonate). Methanol is added to the solution to reduce the viscosity of the ionic liquids and serve as transesterification reactants. Hexane is then added to extract the appropriate quantity of lipids [113]. Because of its quick response time and low vapor pressure, ionic liquid treatment does not require a reactor. By combining two or more ionic liquids, the lipids efficiency can be enhanced because the hydrogen bonds between the ionic liquids and the cell walls are modified.

3.1.4. Electrochemical Method of Lipid Extraction

The electrochemical technique creates current intensity to extract lipids by merging electrical and chemical energy. Electrochemical extraction techniques with various anode materials are employed for effective lipid and protein extraction. Lead oxide and titanium serve as the anode, and stainless-steel serves as the cathode in an electrochemical cell. In an electrolyte solution, the cathode and anode are immersed parallel to one another. The ideal conditions for an electrolytic reaction in a reactor with dimensions of 5 cm wide, 15 cm long, and 17 cm deep are an intensity current of 0.6 A, a recycling flow rate of 394.51 mL/min, and 100 min of electrolysis. Based on particle size, solution concentration, turbidity, and lipids performance, the yield is computed [114].

3.2. Lipid to Biofuel Conversion Techniques

3.2.1. Thermochemical Conversion

Wet microalgae biomass can be converted into biofuel using the thermochemical liquefaction method under low temperature and high pressure. The employment of a catalyst in this process is optional [115]. In such a method, the energy-intensive drying process is eliminated. Biomolecules found in microalgae biomass, including lipids, carbohydrates, and proteins, are converted into crude biofuel by the process of liquefaction. Temperature, pressure, reaction time, catalyst, as well as the natural structure and composition of the microalgal biomass, can all have an impact on how effective the process is [116].

Pyrolysis involves burning biomass at temperatures between 350 and 700 °C without oxygen in order to produce biofuel, syngas, and charcoal [117]. Up to 95.5% of biofuel could be produced using this method [118]. Fast pyrolysis has a lower overall energy usage than slow pyrolysis because of its quick reaction and production of more biofuel than the latter. Additionally, quick pyrolysis produces biofuel with a lower viscosity than slow pyrolysis [119]. Therefore, the quick pyrolysis method is more suited for the manufacturing of liquid biofuel on a larger industrial scale [120,121]. During pyrolysis, microalgae biomass feedstock undergoes several thermo-physical processes, including dehydration, dehydrogenation, deoxygenation, and decarboxylation; these processes break down the biomass into small molecules. Microalgae biomass ingredients are one of the most important elements influencing the variation in the yield and quality of bio-crude. The pyrolysis of the *Chlorella* species of microalgae has a considerable potential for producing high energy content [115]. Additionally, conventional transportation fuel can be simply combined with biofuel produced from biomass microalgae without requiring significant engine adjustments. The pyrolysis of *Spirulina platensis* at 350 °C produced 39.7, 23.8, and 19.2 wt.% of biochar, biofuel, and gases, respectively, according to the experiment described by Jena and Das [122]. According to Shakya et al. [123] nine species of microalgae were subjected to hydrothermal liquefaction at operating temperatures of 280 °C and 320 °C and a pressure of 0.7 MPa. The research verified that the conversion of lipid-rich *Nannochloropsis* at 320 °C produced the maximum yield of bio-crude. The use of heterogeneous catalysts to increase conversion efficiency in the hydrothermal liquefaction of microalgae has been investigated in recent studies [124,125]. Saber et al. [126] used *Nannochloropsis* as the main biomass feedstock for low-temperature hydrothermal liquefaction in their investigation. Three distinct catalysts, including nano-Ni/SiO_2, synthetic zeolite, and Na_2CO_3, were used in the studies, which were carried out at 210, 230, and 250 °C. Nano-Ni/SiO_2 produced

the most biofuel (30 wt%) among these catalysts, followed by Na_2CO_3 (24.2 wt%) and zeolite (24 wt%).

Gasification is the process of heating biomass at relatively high temperatures (800–1000 °C) in low-oxygen environments to produce flammable gas mixtures [127]. Syngas production typically includes a number of common gases, including CO, H_2, CH_4, and CO_2. Despite having a modest energy density (4–6 MJ/m^3), syngas is well suited for use in gas engines or gas turbines for residential heating and cooking [128]. In a study, Zhu et al. [129] carried out an experiment using a 9:1 ratio of microalgae and wood biomass in a co-gasification process. In comparison to using only wood biomass, the co-gasification of microalgae and wood produced higher yields of H_2, CO, and CH_4 by 3–20%, 6–31%, and 9–20%, respectively.

3.2.2. Biochemical Conversion

An effective biochemical method for turning biomass into biofuels is anaerobic digestion. The product of the anaerobic digestion process, which involves bacteria converting the organic biomass of microalgae, is biogas, which is primarily formed of CH_4 and CO_2 with a trace quantity of H_2S. The anaerobic digestion process is often conducted at a steady temperature to ensure the ideal environment for the growth of the bacteria. Mesophilic (30–42 °C) and thermophilic (43–55 °C) temperatures describe the temperature range. Given their popularity and stability in the treatment of various forms of biomass, the majority of anaerobic digesters are built based on the features of their prior operating circumstances [130].

The three primary steps of anaerobic digestion are typically methanogenesis, fermentation, and hydrolysis. Simple sugars are initially produced by hydrolyzing complex polysaccharides found in biomass. Then, alcohols, acetic acid, volatile fatty acids, and minute amounts of H_2 and CO_2 are produced from carbohydrates during the fermentation process. When methanogens are present, the gas mixture further changes into CH_4 (60–70%) and CO_2 (30–40%) [131]. Compared to the lower heating value of the biomass feedstock, the measured energy content of the biogas product is 20–40% higher. Wet biomass with a moisture content of 80–90% has been found to be a suitable fuel for anaerobic digestion [132].

In many investigations, microalgae have been used as the main fuel for the anaerobic digestion process that produces bio-methane. However, the technique only produces very low yields because bacteria cannot degrade the microalgae's outer cell structure. Additionally, because of the low carbon to nitrogen ratio, free ammonia is present, which prevents the creation of methane [133]. The optimum Acetone–butanol–ethanol (ABE) yield under a controlled fermentation procedure is theoretically 0.41 g per gram of sugar, which is a little less than 0:5 g of bioethanol per gram of sugar [134]. According to Chen et al. [135], the high resistance of degraded chemicals coming from the first stages of the fermentation process results in decreased productivity in the generation of biobutanol. Additionally, the conversion of microalgae to biobutanol can only occur in one species (*Clostridium* spp.), which results in a reduced conversion efficiency. Due to *Clostridium* spp.'s ability to break down sugars, the conversion of starch-rich microalga into biobutanol is just as simple as the conversion of bioethanol. The metabolic activities of bacteria result in the biological production of biohydrogen. Compared to other biofuel generation methods, renewable biohydrogen offers a more appealing alternative. Biohydrogen is viewed as a viable alternative fuel since it is thought to be more sustainable, cleaner, and has a reasonably high energy content (142 MJ/kg). There are several biological processes that can be used to create biohydrogen, including photo-fermentation, dark fermentation, and electro-bio-hydrogenation. Regarding viability, sustainability, and energy efficiency, each of these approaches has significant advantages and disadvantages [136].

Researcher interest in microalgae as a potential third-generation biomass feedstock for the creation of renewable biohydrogen has recently increased. Numerous microalgae species, including *Scenedesmus*, *Synechocystis*, *Chlorella*, *Anabaena*, *Nostoc*, and *Tetraspora*, that

possess hydrogenase have been used in studies to produce biohydrogen [137]. Naturally, some types of microalgae can make photo-biological bio-hydrogen by using water and sunlight as sources of energy and electrons, respectively. Additionally, when using microalgae biomass as the organic substrate in the photo-fermentation process with the assistance of photosynthetic microorganisms, hydrogen and carbon dioxide can be obtained [138].

3.2.3. Chemical Conversion

Because microalgae-derived biofuels have a higher viscosity by nature than diesel oils, transesterification must be done on the microalgae oils before using them in engines to reduce their overall viscosity [139]. The direct transesterification approach is thought to be more advantageous for increasing the effectiveness of biodiesel made from microalgae. Notably, in a transesterification process, alcohol serves as both the reactant and the solvent. Methanol is frequently employed in place of other sorts of alcohols in this procedure due to its accessibility and price. The inclusion of a catalyst is also significant since it increases the solubility of alcohol, which is low in various types of oils. [140,141].

Energy content for microalgae-derived biodiesel is between 39 and 41 MJ/kg, which is equivalent to petroleum-based diesel with caloric values of 46 MJ/kg [142]. By using hexane or the supercritical CO_2 extraction process, biodiesel can be produced with a conversion efficiency that is relatively high (70–75 weight percent). In the transesterification of *Nannochloropsis* oculate with the help of CaO and Al_2O_3 catalysts at 50 °C, a very high conversion efficiency rate of 97.5% was reportedly achieved by [143]. Additionally, it was found that the use of Zn, Ti, and Al-based catalysts resulted in a 90.2% conversion efficiency when used in the transesterification of green microalgae at 350–400 °C and 2500 psi pressure [144].

3.2.4. Supercritical Transesterification

A biodiesel conversion process called "supercritical transesterification" involves triglycerides reacting with supercritical alcohols, primarily methanol and ethanol. Due to excessive alcohol consumption at a molar ratio of 42:1, rather than 3:1, as is the case with standard methods, manufacturing costs are considerable. This method has the benefit of converting triglycerides to methyl esters in less than 10 min. Fuel quality is increased as a result of the secondary reaction between unsaturated and esters glycerol at high temperatures [145,146].

3.2.5. Hydrothermal Liquefaction

Hydrothermal liquefaction (HTL) is the process of turning biomass into gas and liquid while keeping a temperature between 150 and 750 °C and a pressure of 5 to 40 Mpa. Although lignin products and waste are employed in the HTL process, less bio-oil is produced than with pyrolysis [147]. Nevertheless, because there is less oxygen present in the reaction with HTL, the quality is better than pyrolysis. The high heat transfer rate and reaction at 90% moisture content of this method are benefits [148]. Following the removal of oxygen, HTL crude can be used directly for transportation [108]. The characteristics of biodiesel are evaluated in accordance with ASTM standards to determine its suitability for transportation. Several characteristics of microalgal biodiesel in comparison to other oils [149,150]. Supplementary Table S4 shows the different lipid conversion methods. The entire microalgae biomass is broken down and transformed in hot compressed water via hydrothermal liquefaction (HTL), which eliminates the need for drying. A biocrude oil is produced as the principal by-product, along with gaseous, aqueous, and solid by-products. Due to the high levels of nitrogen in proteins and chlorophyll, this biocrude oil may produce high NOx emissions during combustion but has low sulfur emissions. It also has a low ash content, which reduces particle emissions [151–153].

3.3. Biodiesel Purification

Wet and dry washing are used to purify biodiesel [154]. Even though these procedures produce high-quality biodiesel that satisfies the requirements of both ASTMD6751 and EN14214 standards, they have a number of drawbacks that restrict their use and pose a challenge for the commercialization of biodiesel. In actuality, the downsides of the biodiesel water-washing process include the usage of significant volumes of water, yield loss, high energy consumption, higher cost, and wastewater disposal (20–120 L of wastewater per 100 L of biodiesel) [155]. Dry-washing procedures were created to get over these restrictions. Examples include the purification of crude biodiesel using activated carbon [156], cellulose [157], magnesium silicate (magnesol) [158], and silica [159]. Nevertheless, dry washing is discouraged because it generates sludge with non-recyclable adsorbents, which also creates a significant disposal and environmental issue. Additionally, biodiesel produced through dry-washing purification utilizing magnesium silicates and ion-exchange resins does not meet the EN14214 standard's requirements for a methanol limit [160].

3.4. Quality of Microalgal Biofuel

3.4.1. Physical and Chemical Properties of Microalgal Biodiesel

The physical and chemical characteristics of microalgae biodiesel and diesel are compared to those of European biodiesel standards (EN14214) [161], which have been the subject of several studies. Review results indicate that all measured parameters, with the exception of cetane quantity, meet EN14214 requirements. Diesel fuel's ignition quality is indicated by its cetane rating; if it is low, the ignition delay time will lengthen, which results in incomplete combustion because of a constrained combustion time [161].

3.4.2. Effect of Injection Timing on Combustion Characteristics

Jayaprabakar et al. [161] investigated the effect of injection timing on the combustion characteristics of a compression ignition (CI) engine running on a B20 blend of 80% diesel and 20% biodiesel. Due to its reduced volatility compared to diesel, the heat generated during combustion is not used as effectively. The findings indicate that the physical and chemical features of algal biodiesel are comparable to those of conventional biodiesel, and based on its combustion qualities, B20 can be used as a superior fuel to diesel [162].

3.4.3. Droplet Combustion Characteristics

Based on relative flame position, combustion rate, and soot aggregates to droplet surface, conventional diesel, hydro processed renewable diesel fuel (HRD), and R50 (50/50 of HRD and DF2) were examined. In terms of sooting and chemical differences, the HRD droplets were comparable to DF2 and R50 blends. The sooting propensity was discovered to be DF2 > R50 > HRD (high to low). Among all the mixes, DF2 showed the highest sooting tendency; its high aromatic content (27%) may be to blame. Based on the droplet combustion characteristics, the analysis indicated that HRD has significant potential as a replacement for conventional fuel or as an additive [163].

A Ricardo E6 (indirect injection) diesel engine has been tested with two forms of microalgae oil, ME0.2 and ME0.1 (in transesterification, 20% and 10% methanol in comparison to oil). The engine ran smoothly on algal oil methyl ester since it shared many characteristics with diesel fuel. When compared to diesel fuel, ME0.1 performed better than ME0.2, but the engine torque was decreased and the combustion noise was slightly increased [162]. These issues can be resolved by modifying the engine's design characteristics, such as the injection timing and compression ratio.

3.4.4. Engine Performance and Emissions

A heavy-duty diesel engine was fueled with two B100 biodiesel samples, dubbed BA (the algal *Chlorella* variabilities) and BJ (*Jatropha curcas*). Performance and emission parameters were investigated over the 600-s long urban, rural, and highway modes that make up the European Transient Cycle (ETC). According to the experimental findings,

using B100 (BA and BJ) as fuel results in much higher NOx emissions while producing less particulate matter (PM), hydrocarbons (HC), and carbon monoxide (CO) than fossil fuels. Because diesel has a low calorific value, BA and BJ had greater brake specific fuel consumption (BSFC) values than diesel. Compared to BJ, an engine running on BA produced 17% less NOx and 6% less BSFC. According to Violeta Makareviciene's research, mineral diesel fuel and B30 algae methyl ester showed a thermal efficiency difference of 2.5–3%. According to the investigation, biodiesel made from *Chlorella* varieties of algae can be used as a substitute fuel in CI engines [164–166].

4. Techno-Economic Analysis

By taking into account several factors, including capital and operational expenses as well as numerous hazards (social, environmental, etc.) related to manufacturing technologies and processes, TEA is used to examine the economic viability of a process or product [167]. With the help of a TEA, it is possible to pinpoint the economic factors that have the greatest impact on microalgae biodiesel prices and take action to bring them down. Although multiple TEA have been reported by various academics to assess the viability of biofuels from an economic standpoint [168], the outcomes are very inconsistent. For example, Richardson et al. [169] estimated the cost of microalgal biodiesel was as high as $31.36 per gallon while Nagarajan et al. [170] estimated that the cost was $2.20 per gallon. Taking into account the process limits, the variability of various assumptions employed in cost calculations, etc., leads to this significant difference. The cost of producing biomass is the main determining factor [168]. The dry and wet approaches were used by Gautam et al. [171] to conduct the TEA of microalgal biodiesel synthesis. According to their reports, the price ranged between $1.00 per kg and $25.0 per kg and $1.00 per kg and roughly $12.0 per kg, respectively. According to reports, variances in harvesting and separation methods, plant capacity, product purification, etc., were to blame for the cost variations. According to many published investigations, the price of microalgal lipids must be less than $2.80 a gallon in order to compete with the price of existing conventional diesel [172]. Nevertheless, the cultivation method significantly affects the price of lipids produced by microalgae, algal strain, and productivity; the vast majority of research has concurred that high growth rate, direct transesterification, and lipid extraction from wet biomass would enhance the process's economic viability. Additionally, it is not profitable to produce algal oil for biodiesel alone. Microalgae should be looked into as a whole feedstock to produce a variety of co-products and biofuel in order to make microalgal biofuel economically viable [173]. In this regard, [174] demonstrated *Phaeodactylum tricornutum's* composition, which includes 5.19 wt% biosilica, 0.86 wt% fucoxanthin, 9.08 wt% lipids, 38.40 wt% proteins, and 7.85 wt% carbs. The economics of the microalgal bioproducts are improved by valorizing all these substances found in the microalgae using a biorefinery technique.

Mustapha et al. [175] successfully decreased the cost of biodiesel production from microalgae to 1.7 $/kg. the authors converted the biomass oil to biodiesel using a lipid extracted algae derived catalyst method that de-oiled. In a similar study, Sawaengsak et al. [176] investigated the aquatic feed using the de-oiled algal biomass; the results showed that the cost of biodiesel produced from photobioreactors was 6.7 $/L, whereas the use of a raceway for biodiesel production can produce biodiesel with price of 2.03 $/L. The cost of palm oil-based biodiesel production in Malaysia is more 2.39 $/L, which is higher than the cost of microalgal-based biodiesel. In addition, the cost of fossil diesel in Malaysia is a bit lower than palm oil-bio diesel [177]. Soyabean oil is the main source for biodiesel production in Brazil, which is the second highest producer of biodiesel in the word. You et al. [178] found that the price of soyabean produced biodiesel was 2.95$/L. On the other hand, the waste cooking oil produced biodiesel cost 1.93 $/L according to Marchetti et al. [179]. Other feedstocks, such as castor oil and rapeseed oil [180], were used to produce biodiesel; however, the cost of the production was high (5.9 $/L for castor oil and 7.72$ for rapeseed oil).

5. Environmental Impact of Microalgae Biofuel Production

Impacts on the environment measure how different routes respond to stressors including resource depletion, acidification, and global warming. These impact analyses show which industries are more likely to cause stress and which should shift to more ecologically friendly options [181]. As a feedstock, microalgae can reduce carbon emissions by capturing carbon from the atmosphere. However, the process of turning microalgae into goods is equally important because the reduced emissions must be translated along the entire value chain. A rotating algal biofilm reactor's lifespan has been studied by Barlow [182] for the purpose of producing bio-oil by hydrothermal liquefaction. According to the study, the reactor cycle, biomass productivity, and bio-crude yield all have a significant impact on the likelihood of global warming. One MJ of bio-oil produces 80 g of CO_2 in the "business as usual" scenario compared to 44 g of CO_2 in the "optimal scenario" with improved productivity and crude yield. In a life cycle assessment (LCA), the Net Energy Ratio (NER), which describes the proportion of energy conserved to energy needed in a process, is a crucial factor. The NER can go up or down depending on the reactor used for algae cultivation. Raceway Pond, for instance, has a NER of 8.3, whereas flat reactors have a NER of 4.5 and tubular reactors have a NER of 0.2 [181]. The whole energy balance is impacted by this net energy ratio, which in turn influences other environmental impact factors like global warming, acidification risk, and resource potential. The environmental parameter known as global warning potential provides information about a system's emissions. Typically, the potential for global warming is stated in equivalent units of CO_2. To express it in a common phrase, the potential of the various greenhouse gases is converted to CO_2 equivalency. Algal biorefinery is a carbon sequestration technique that reduces overall emissions. However, biomass drying requires a significant amount of energy, which lessens its adverse effects. For instance, the global warming potential (GWP) of algae fuel produced through pyrolysis is 210 gCO_2/MJ while the net emission from hydrothermal liquefaction is only 10 gCO_2/MJ. The net emissions of ordinary diesel, in contrast, are 15 gCO_2/MJ [182].

6. Recommendation and Future Prospective

Even though macro- and microalgae are potentially useful for biotechnological, industrial, and environmental applications, the cost of producing algal fuel is still higher than that of fossil fuels. The cost of nutrients has been reduced by using wastewater and flue gases, but other mechanical equipment and technologies are expensive. In other words, the fundamental processes used for the manufacturing and marketing of algae biofuels continue to be a significant barrier. Similar to this, using chemicals, technology, electricity, and labor effectively to produce microalgal biofuel presents significant challenges. Microalgae culture in closed and open reactors with an ideal pH, temperature, and light will support the rapid doubling of microalgae producing high biomass. A more effective method to increase the compound of interest in the microalgae will be developed through the alteration of the growing state, nutritional stress, and physical modification. In addition, the poor productivity of these compounds and metabolites in microalgae limits the production of value-added goods, including pigments, enzymes, proteins, and other polysaccharides. As a result, strategies for maximizing the value of algal products must be determined. The complicated extraction, drying, harvesting, and processing procedures limit the use of algae cells as a feedstock for making biodiesel. Therefore, researchers must concentrate on finding a solution for these drawbacks and increasing production. Solar light, carbon dioxide, water, and nutrients, including nitrogen, sulfur, phosphorus, and iron, are all necessary for algal development. However, as climatic conditions change, providing these nutrient sources and environmental conditions is challenging in terms of sustainability. Another significant difficulty factor is the cost. The expense of growing and collecting algae is higher. Using wastewater for the cultivation of algae species is one way to cut costs.

Summarizing the considerations, it is clear that future research must concentrate on bridging the knowledge gap and addressing issues, such as the necessity for an adequate cultivating system given that biomass productivity and lipid accumulation are incompatible.

For algal biodiesel to be more economically viable, the cultivation system must be improved to enable the production of a significant amount of lipid-rich algae biomass, and more effort and equipment are needed for harvesting methods.

7. Conclusions

In this review, the latest update regarding the application of microalgae for biofuel production, including cultivation, harvesting, and lipid extraction and conversion, was discussed. Environmental factors such as pH, CO_2 concentration, light intensity, and temperature have a significant impact on microalgal cultivation. The main difficulties with using physical methods to dewater a larger volume of microalgal growth are the high energy and longer time requirements, which emphasize the necessity for an efficient harvesting process. The main advantages of microalgal biofuels are their high ability to fix CO_2, environmental remediation potential and green technology for sustainable energy production. Nevertheless, microalgal biofuel production processes, such as cultivation, harvesting, and lipid conversion, are energy costly, resulting in a high cost of the overall process. Therefore, to increase the sustainability of microalgae application for biofuel production, additional research into co-cultivation systems, innovative green harvesting techniques, high lipids extraction methods, and optimizing the lipid extraction and converting process should be implemented.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en16031316/s1. Table S1: Environmental variables affecting growth of microalgae biomass production (optimal condition for biomass). Table S2: Comparison of different algal cultivation methods and systems. Table S3: A comparison of different microalgal harvesting technique. Table S4: Biofuel conversion processes. References [183–216] are cited in the Supplementary Materials.

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