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# Microalgal Feedstock for Biofuel Production: Recent Advances, Challenges, and Future Perspective

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**Abstract:** Globally, nations are trying to address environmental issues such as global warming and climate change, along with the burden of declining fossil fuel reserves. Furthermore, countries aim to reach zero carbon emissions within the existing and rising global energy crisis. Therefore, bio-based alternative sustainable feedstocks are being explored for producing bioenergy. One such renewable energy resource is microalgae; these are photosynthetic microorganisms that grow on non-arable land, in extreme climatic conditions, and have the ability to thrive even in sea and wastewater. Microalgae have high photosynthetic efficiencies and biomass productivity compared to other terrestrial plants. Whole microalgae biomass or their extracted metabolites can be converted to various biofuels such as bioethanol, biodiesel, biocrude oil, pyrolytic bio-oil, biomethane, biohydrogen, and bio jet fuel. However, several challenges still exist before faster and broader commercial application of microalgae as a sustainable bioenergy feedstock for biofuel production. Selection of appropriate microalgal strains, development of biomass pre-concentrating techniques, and utilization of wet microalgal biomass for biofuel production, coupled with an integrated biorefinery approach for producing value-added products, could improve the environmental sustainability and economic viability of microalgal biofuel. This article will review the current status of research on microalgal biofuels and their future perspective.

**Keywords:** microalgae; biofuels; techno-economic analysis; environmental impact; bioenergy

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## 1. Introduction

Energy has played a significant role in developing human civilizations [1]. Nations have utilized non-renewable energy sources such as coal, oil, and gas to maintain their accelerated growth [2]. According to the reported models, fossil-derived energy sources are expected to reach maximum utilization by the year 2050 and will begin to show a decline by 2075 [3,4]. With the increasing energy crisis, countries are now looking for alternative and renewable energy solutions. Furthermore, over the next few decades, countries such as China and India have set targets to reach zero carbon emissions to mitigate climate change [5,6]. Initiatives such as the Paris Agreement and Kyoto Protocol have been undertaken to tackle the issues of climate change and global warming; still, it is projected that the global temperature might rise by 3 °C, thereby making climate change mitigation more challenging [7]. Therefore, various forms of bioenergy source are currently being developed and implemented globally to tackle the challenges of depleting non-renewable fossil fuel-based energy sources for achieving net zero carbon emissions. These bioenergy sources include but are not limited to microalgae, wood (or forestry crop) and wood processing residues (e.g., firewood and wood chips), agricultural crop residues (e.g., rice, corn, wheat, soybeans, and macroalgae), dedicated energy crops (e.g., switchgrass,

miscanthus, fast-growing willow, and poplar), municipal solid waste (e.g., paper, cotton, and plastic), animal waste (e.g., poultry litter and pig manure), sewage, and industrial waste (e.g., black liquor and peelings and scraps from fruit and vegetables) [8–11].

Terrestrial plants and photosynthetic microorganisms such as microalgae are currently being explored as feedstocks to develop biofuels and achieve carbon neutrality [12,13]. While efforts are underway to increase energy efficiency and capacity, compared to wind, solar, and hydropower renewable energy sources, liquid biofuels from terrestrial biomass and microalgae can readily be translocated, stored, and directly used as engine fuels [14]. To date, biofuel production has been divided into 1st (1G), 2nd (2G), and 3rd (3G) generation biofuels [15]. The 1st and 2nd generation biofuels such as bioethanol, biodiesel, and biomethane as biofuel products were primarily produced from terrestrial plants, woody biomass, and agricultural and municipal solid waste [16]. Between 2013 and 2015, approximately 75% of bioethanol was produced from sugarcane, corn, and maize crops. At the same time, 81% of biodiesel, a 2G biofuel, was reported to be produced from vegetable oil extracted from various edible and non-edible seed-bearing plants. Furthermore, the production of 1st generation (1G) bioethanol has been reported to not only utilize agricultural lands but also adversely impact biodiversity, change land use, and emit pollutants and carbon dioxide ($CO_2$). Due to the abovementioned issues, the focus shifted towards producing 2nd generation (2G) biofuel from feedstocks that do not directly compete with food. For example, various oil sources such as waste cooking oil and non-edible oil sources (Jatropha, Karanja, etc.) were explored for biodiesel production; similarly, efforts were made to produce bioethanol from lignocellulosic compounds [12]. Although 2G biofuel was produced from non-food crops, it required large agricultural land to grow oil-bearing crops. Utilizing large amounts of agricultural space to produce biodiesel could push developing countries towards poverty again, raising the food vs. fuel debate. Therefore, feedstocks that do not give rise to food vs. fuel issues and have minimal adverse environmental impacts are currently being explored for producing 3rd generation (3G) biofuel. Microalgal biomass is among the potential 3G biofuel feedstocks. Microalgae are mostly microscopic photosynthetic microorganisms that can produce biomass several times higher than any other terrestrial plant [17]. Several microalgal strains have the ability to capture $CO_2$ from the atmosphere and flue gases [18]. Microalgae have three times higher solar conversion efficiencies compared to terrestrial plants [19]. Microalgae can utilize various forms of carbon in phototrophic, autotrophic, and heterotrophic modes of cultivation [20]. Microalgae have a unique ability to convert both inorganic and organic carbon to biomass. Microalgae biomass is rich in proteins, lipids, and carbohydrates. Marine or brackish microalgal strains have a minimal freshwater footprint and can be cultivated on non-arable land in extreme climatic conditions. Furthermore, some microalgae strains, such as *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Dunaliella* sp., *Scenedesmus dimorphus*, *Coelatrella* sp. and *Spirulina* sp. could accumulate high amounts of carbohydrate, lipids, and protein making them ideal feedstocks for biofuel production [21,22].

Due to the advantages mentioned above, microalgae have been studied as feedstock for producing various biofuels, such as bioethanol, biodiesel, biocrude oil, bio-jet fuels, pyrolytic bio-oil, biohydrogen, biomethane, etc. Although microalgae could be cultivated in phototrophic, mixotrophic, and heterotrophic modes, the cost and energy requirement of biomass production in these modes could be prohibitively high [23]. Hence, extracting high-value metabolites from microalgal biomass, regardless of the mode of cultivation, could support biofuel production from its leftover biomass. This review will evaluate the potential of whole microalgae biomass and its specific metabolites as feedstocks for producing (3G) biofuels.

One of the objectives of this study was to compare the processing technology and operating conditions involved in producing seven selected microalgal biofuels. Another objective was to make a comparative analysis of the net energy ratio (NER), carbon emissions as $CO_2$ eq./kg biofuel, and techno-economics. Finally, the associated challenges and future prospects of the selected microalgal biofuels were discussed.

## 2. Microalgal Biofuels

Microalgae could be cultivated in open raceway ponds and closed photobioreactors [24,25]. Microalgae can be cultivated in seawater, freshwater, brackish, and wastewater and can produce different macromolecules such as proteins, lipids, and carbohydrates in various proportions [26,27]. Whole microalgal biomass or their extracted macromolecules can be converted to biofuels, such as bioethanol, biodiesel, biocrude oil, bio-jet fuel, pyrolysis oil, biomethane, biohydrogen, etc. [28]. The following sections describe various production techniques and processing parameters applied to microalgal feedstock for producing the selected biofuels.

### *2.1. Bioethanol*

Bioethanol is produced by alcoholic fermentation, which uses algal biomass containing sugars, starch, or cellulose [29]. In this process, the biomass is broken down, and the starch is turned into sugars (known as saccharification/hydrolysis), which are then degraded by yeast cells or bacteria to produce ethanol in anaerobic conditions (Figure 1). Other by-products are $CO_2$ and water in alcoholic fermentation. Purifying the diluted product (8–16% ethanol) is a major step in ethanol industries to eliminate other impurities such as methanol, butanol, 3-methyl butanol, and acetaldehyde [30]. Currently, most of the ethanol production plants operate using first and second-generation feedstocks, which brings into question their sustainability.

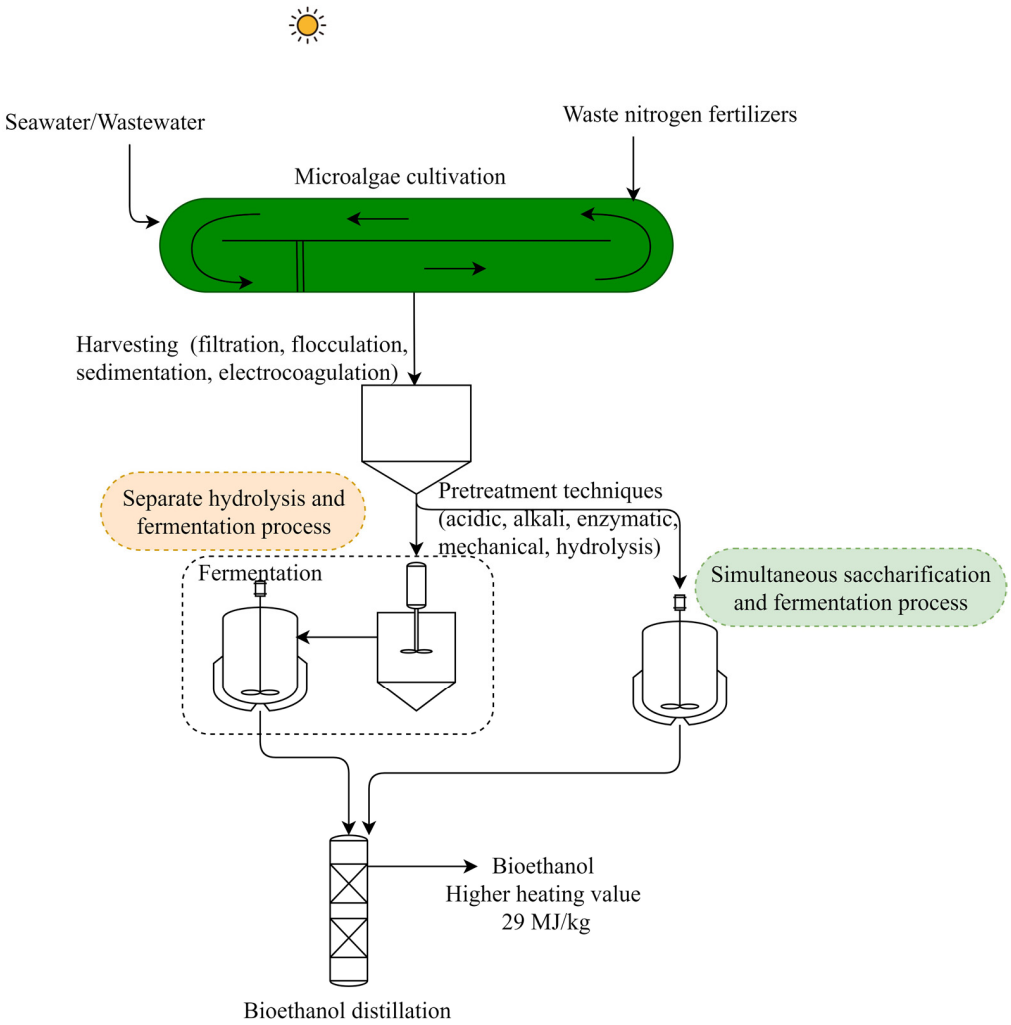



**Figure 1.** Process flow diagram for bioethanol production from microalgae.

On the other hand, microalgae feedstocks are promising. As shown in Table 1, bioethanol production from various microalgae biomass ranges from 0.07 to 0.5 g $g^{-1}$. Indeed, the production yield depends on the carbohydrate content in the microalgae biomass. Microalgae such as *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella vulgaris*, *Dunaliella* sp., and *Scenedesmus dimorphus* could reach up to a 69.7% carbohydrate content to make the alcoholic fermentation ideal [21,22]. *Zymomonas mobilis* (bacteria) and *Saccharomyces cerevisiae* (yeast) are the major microbes that can ferment starch to ethanol [31]. Nevertheless, algae feedstocks often require rigorous pretreatment (chemical or biological) before processing since the microbes cannot utilize polysaccharides directly. The hydrolysis of the complex substrate can be either carried out by separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) [32]. In SHF, biomass hydrolysis and fermentation are conducted in different reactors to control each unit operation separately to achieve the desired result [33]. The pretreatment step primarily comprises an acid treatment, involving the addition of a strong acid, such as $H_2SO_4$, often coupled with heating in an autoclave or treatment with hydrolytic enzymes such as α-amylase amyloglucosidase, cellulase, β-glucosidase or organic solvent such as chloroform-methanol mixtures or supercritical $CO_2$ [34]. Furthermore, when $H_2SO_4$ was used to pretreat two different *Leptolynbia* sp. (Table 1), the difference in bioethanol yields was minimal, whereas the bioethanol yield for *Chlorococcum* sp. pretreated using supercritical $CO_2$ was lower than for *Chlorococcum* sp. treated using $H_2SO_4$. Interestingly, for *Scenedesmus* sp. biomass, pretreatment by organic solvent and enzymatic mixture provided higher bioethanol yield over $H_2SO_4$ pretreatment; this indicated that the organic solvents could have effectively disintegrated the microalgal cell wall of *Scenedesmus* sp. (Table 1). The resulting hydrolysates are then subjected to fermentation at a temperature commonly ranging from 25 to 30 °C, in the presence of fermenting microorganisms, such as *Saccharomyces cerevisiae*. However, other pretreatment techniques and their respective fermentation conditions were explored for bioethanol production [34–44].

**Table 1.** Bioethanol production from various microalgae strains.

| Microalgae Feedstock | Pretreatment Process Parameters | Fermentation Process Conditions; Microorganism | Bioethanol Yield g $g^{-1}$ (dry wt Basis) | Reference |
|---|---|---|---|---|
| *Scenedesmus dimorphis* | Organosolv (2:1), 24 h, 750 rpm, enzymatic hydrolysis | 150 rpm, 34 °C, *S. cerevisiae* | 0.266 | [34] |
| *Chlorella vulgaris* | 200 g $L^{-1}$, $H_2SO_4$ at 120 °C for 20 min | 200 rpm, 30 °C; *S. cerevisiae* | 0.214 | [35] |
| *Tetraselmis* sp. | Chemo-enzymatic hydrolysis and pH 5, 60 °C | 150 rpm, 30 °C, 48 h, *S. cerevisiae* | 0.314 | [44] |
| *Chlorella* sp. | 3% $H_2SO_4$, 121 °C for 20 min | 30 °C, 150 rpm, 20 h; *S. cerevisiae* | 0.4 * | [36] |
| *Leptolyngbia* sp. | 1.5 N $H_2SO_4$, 0.8 bar, 3 h | 250 mL, 30 °C, 4 h, 150 rpm *S. cerevisiae* | 0.113 | [37] |
| *Leptolyngbia valderiana* | 1:15 biomass to liquid, $H_2SO_4$, $MgSO_4$ at 121 °C for 20 min. | 200 rpm, 80 h, 30 °C; *S. cerevisiae* | 0.16 | [38] |
| *Chlorococum* sp. | $H_2SO_4$, pH 7, 160 °C | 30 °C, 48 h, 200 rpm | 0.52 | [39] |
| *Chlorococum* sp. | Supercritical $CO_2$ | 200 rpm, 60 h, 30 °C, *S. bayanus* | 0.35 | [40] |
| *Desmodesmus* sp. | 10% solid loading, 120 °C, 30 min, $H_2SO_4$ | 28 °C, 120 rpm, 30 h, *S. cerevisiae* | 0.24 | [41] |
| *Scenedesmus acuminatus* | 2N $H_2SO_4$, autoclaving, pH 5.5 | 200 rpm, 30 °C, 80 h, *S. cerevisiae* | 0.12 | [42] |
| *Tetraselmis* sp. | 0.75% Sodium hydroxide for 10 min | 48 h, 30 °C, *S. cerevisiae* | 0.073 | [43] |

* g $g^{-1}$ carbohydrate.

A combination of chemical and enzymatic pretreatment of *Tetraselmis* sp. could also improve bioethanol yield (Table 1). The hydrolysates of microalgal biomass produced after

various pretreatments were then used to produce bioethanol using *Saccharomyces cerevisiae* using different process parameters (i.e., temperatures, rpm, time duration; Table 1).

For example, the biomass of *Chlorella* sp. was firstly hydrolyzed by glucoamylase (enzymatic saccharification) at 65 °C, and then the hydrolysate was subjected to yeast fermentation using *Saccharomyces cerevisiae* at 30 °C for bioethanol production [45]. A maximum of 0.116 g of ethanol was obtained from 1 g of algal biomass. Similarly, hydrolysis of algal biomass can be done by acid addition. In the pretreatment step, 2% (*v*/*v*) $H_2SO_4$ was used at a temperature of 121 °C for the hydrolysis of de-oiled mixed microalgae [46], and then the extract was separately utilized as substrate for ethanol fermentation using *Saccharomyces cerevisiae* at 30 °C, which exhibited an ethanol yield of 0.116 $g.g^{-1}$ biomass [46]. On the other hand, hydrolysis and fermentation have identical operating conditions in SSF; eventually, both are processed in a single unit. For instance, Huang et al. (2020) utilized recombinant *Saccharomyces cerevisiae*, which was capable of producing cellulases and amylases that simultaneously saccharified pigment-extracted *Chlamydomonas* sp. biomass and fermented the sugar to ethanol at 30 °C [47]. By applying a consolidated bioprocessing technique, the bioethanol yield from the leftover biomass was enhanced by 31% [47].

### 2.2. Biodiesel

Biodiesel, commonly known as fatty acid methyl esters (FAME), is synthesized by esterification or transesterification process from edible or non-edible vegetable oils. Different types of feedstocks are used in various countries for producing biodiesel; for example, soybean oil, palm oil, and rapeseed oils are used to produce biodiesel in the USA, Malaysia, and European countries, respectively [48–50]. In countries like India, non-edible oils, such as Jatropha, Karanja, Mahua, and neem oils, have been used for biodiesel production [51]. The production of biodiesel from edible or non-edible oil, and overexploitation of edible oils, and agricultural land use for biodiesel production have been linked to a food crisis in developing countries [52]. Therefore, microalgae have been extensively researched as one of the potential feedstocks for biodiesel production (Figure 2).

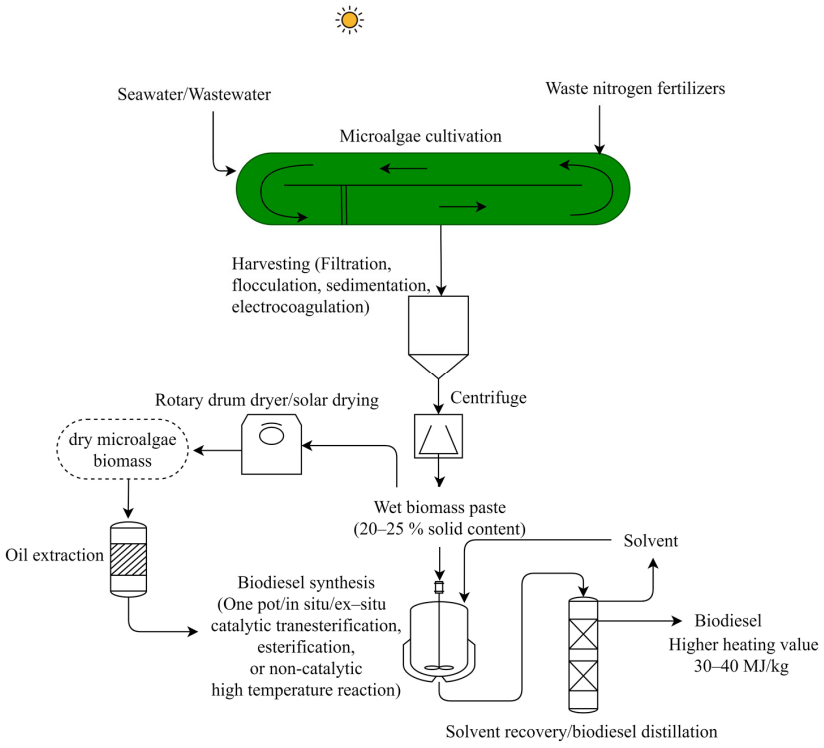



**Figure 2.** Process flow diagram for biodiesel production from microalgae.

The conventional method for producing biodiesel from microalgae includes selecting microalgae strains, such as *Nannochloropsis* sp., *Chlorella sorokiniana*, and *Chlorella protothecoides*, with lipid content ranging from 15 to 40% [53]. In the conventional ex-situ transesterification process, crude oil is extracted from microalgal biomass, which is later converted to biodiesel. *Chlorella protothecoides* oil was converted to biodiesel at 60 °C in the presence of an alkali catalyst (e.g., KOH), and fatty acid methyl esters produced had an unsaturated content of >90%. The biodiesel produced satisfied the required diesel fuel standard, and a higher degree of unsaturation imparted excellent cold flow properties to microalgal biodiesel [54]. Another study demonstrated the use of biocatalyst lipase GH2, derived from a recombinant fungus. Oil from *Chlorella vulgaris* was first extracted and later mixed with a biphasic solvent system containing ethanol, methanol, n-hexane, and biocatalyst lipase GH2; the mixture was kept stirring at 30 °C for 24 h. The biodiesel yields as FAME and fatty acid ethyl esters (FAEE) were >90%, indicating that biodiesel could be feasibly produced at lower reaction temperatures with higher conversion efficiencies [55]. Nanocatalyst, such as $Ca(OCH_3)_2$, has also been studied to convert *Tetraselmis indica* oil to biodiesel [56]. Nanocatalysts could act as heterogenous and homogeneous catalysts and remain unaffected by the presence of free fatty acids (FFA) and water while enhancing the transesterification reaction rate. In a single-step reaction, the nanocatalyst could successfully convert microalgae oil with varying FFA content to biodiesel [56]. Although the successful conversion of microalgal oil is well documented, extracting microalgae oil involves energy-intensive dewatering and drying steps; therefore, alternative wet biomass processing could reduce the energy required for producing microalgae-based biodiesel [57–61]. Table 2 shows recent studies utilizing wet microalgae biomass for biodiesel synthesis.

**Table 2.** Biodiesel production from various microalgal strains in wet form.

| Microalgae Feedstock | Biomass and Reaction Parameters | Reaction Technique | Biodiesel Conversion (%) | Reference |
|---|---|---|---|---|
| *Chlorella pyrenoidosa* | Wet biomass with 77% water content, methanol to biomass 8:1. $H_2SO_4$, 90 °C, 30 min. | Microwave-assisted | 86 | [57] |
| *Nannochloropsis* sp. | Wet biomass with 80% water content, solvent system methanol and ionic liquid, 65 °C, 15 min | Microwave-assisted | 36.7 | [58] |
| *Aurantiochytrium* sp. | Wet biomass with 40% water content, solvent ethanol, catalyst potassium carbonate, 60 °C, 60 min | Ultrasound-assisted | 80 | [59] |
| *Nannochloropsis* sp. | Wet biomass with 80 % water content, ethanol, and $H_2SO_4$, 150 °C, 120 min, 15 MPa | Supercritical carbon dioxide | 25 | [60] |
| *Spirulina platensis* | Wet biomass with 40 % water content, methanol hexane, 300 °C, 30 min, 67 bar | Supercritical methanol | 99 | [61] |

In-situ biodiesel production from microalgae biomass has been widely studied. The ionic liquid could simultaneously disrupt microalgal cells and perform transesterification. For example, an ionic liquid tetra butyl phosphonium formate could process wet *Chlorella vulgaris* biomass (40% moisture) to biodiesel with 98% conversion efficiency of the fatty acids [62]. Furthermore, the study demonstrated repeated reusability of the ionic liquid. In a one-pot study, biodiesel was produced from the wet *Nannochloropsis oceanica* (65% moisture) in the presence of methanol and sulfuric acid; the technique provided a 91% FAME yield [63]. In-situ biodiesel production eliminates the need for the microalgal biomass drying and oil extraction step, which could improve the economic viability of microalgal biodiesel production.

### 2.3. Biocrude Oil

Over the past few decades, hydrothermal liquefaction (HTL) has emerged as a promising thermochemical technique for converting different types of biomass to biocrude oil [64]. Several feedstocks, such as lignocellulosic waste, municipal solid waste, food waste, etc., were studied for biocrude production. However, microalgae biomass as a feedstock for HTL has several advantages, such as higher biocrude yield and better quality (e.g., calorific value) [24]. The advantage of the HTL technique for biocrude production is that wet biomass slurry can be used directly as an HTL feedstock, thereby eliminating the need for energy-intensive drying steps [65].

Almost a decade ago, research began into the production of biocrude using hydrothermal processing of microalgae (Figure 3); the HTL reactions were conducted at temperatures ranging from 300 to 350 °C in the presence of organic and alkali catalysts [64,65]. Similarly, in the initial stages, the research on the HTL process was primarily focused on biocrude production from different microalgae feedstocks [64]. Later, extensive research focused on (i) enhancing the biocrude yields from microalgae biomass in both the batch and continuous modes by utilizing different catalysts [65]; and (ii) improving the biocrude oil quality by using catalytic hydrotreatment technique for subsequent hydrodeoxygenation and hydrodenitrogenation.

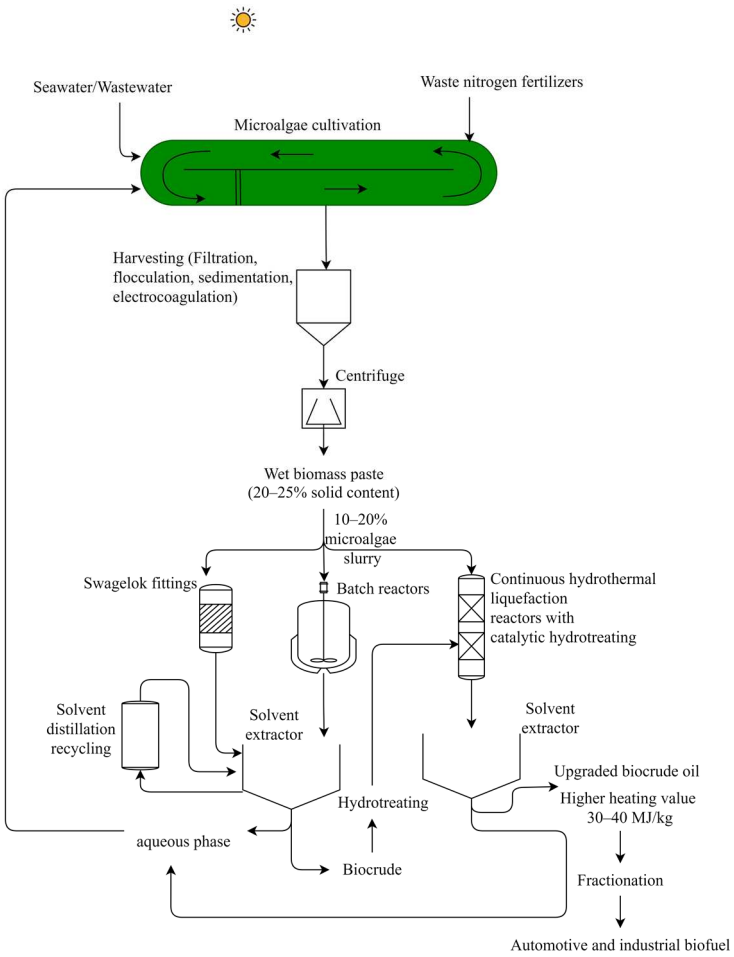



**Figure 3.** Process flow diagram for biocrude oil production from microalgae.

In the HTL process, under sub and supercritical conditions, the water acts as a solvent and a catalyst that can effectively convert wet biomass to biocrude oil with other byproducts such as biochar or hydrochar, aqueous phase, and gas [66]. Hydrothermal liquefaction reactions were mainly conducted in batch-scale reactors and Swagelok fittings, with working

volumes ranging from 0.01 to 2 L [65,67]. Several other studies have reported continuous HTL processes for biocrude production coupled with hydrotreatment to upgrade the quality of biocrude oil [68]. In the HTL technique, biological macromolecules (proteins, lipids, and carbohydrates) present in biomass are converted into various hydrocarbons and organic compounds that together form biocrude oil. The biocrude formation occurs due to dehydration, condensation, hydrolysis, deamination, etc.; these reactions take place in the presence of sub and supercritical water, leading to the formation of hydrocarbons such as alkanes, alkenes and aromatics with varying degrees of oxygenated and nitrogenous compounds [69–71].

Table 3 shows the various microalgal feedstocks utilized for producing biocrude oil, with their respective yields, HTL processing conditions, and the quality of biocrude oil. The H/C and O/C ratios (Table 3) are vital in determining the quality of biocrude oil. The microalgal biocrude oil quality can be improved by increasing H/C ratio and lowering the O/C ratio through hydrotreating the biocrude, thereby lowering the heteroatoms (i.e., O and N content) and increasing the higher heating value (HHV) of biocrude oil [25].

**Table 3.** Biocrude oil production from batch HTL process of microalgae strains.

| Microalgae Feedstock | Solid Content (%) | HTL Processing Conditions | Biocrude Oil Yield (%) | H/C | O/C | References |
|---|---|---|---|---|---|---|
| *Tetraselmis* sp. | 10 | 275–350 °C, 30 min | 31 | 1.57 | 0.119 | [72] |
| *Chlorella* sp. | 20 | 320 °C, 1 MPa, 320 °C | 33.8 | 1.5 | 0.28 | [73] |
| *Nannochloropsis gaditana* | 16.6 | 374 °C, 200 rpm, 239–374 °C, 60 min | 15 | 1.45 | 0.23 | [74] |
| *Neochloris* sp. | 15 | 350 °C, 60 min | 36 | - | - | [75] |
| *Botryococcus* sp. | 15 | 350 °C, 60 min | 40 | - | - | [75] |
| *Spirulina platensis* | 9 | 315 °C, 15 min | 20.96 | 1.36 | 0.17 | [76] |

### *2.4. Pyrolysis Oil*

Pyrolysis is a commercially well-established thermochemical technique that converts organic biomass to biofuel or bio-oil. Pyrolysis is carried out in the absence of oxygen at high temperatures ranging from 300 to 600 °C, wherein the chemical bonds between the organic material are broken down, resulting in bio-oil [77]. Presently, pyrolysis is considered the cheapest and lowest-cost biofuel-producing technology. However, pyrolysis products contain large amounts of oxygenated and nitrogenous compounds, reducing the energy density or the calorific value of the bio-oil [78].

Recently, microalgae have been used as a feedstock for pyrolysis for producing bio-oil (Figure 4). For example, *Nannochloropsis* sp. biomass was subjected to pyrolysis at 600 °C in the presence and absence of a zeolite catalyst; bio-oil yield in the absence and presence of a catalyst was 58.1 and 45.3%, respectively [79]. In catalyst-aided pyrolysis, gas and water vapor content increased, lowering the bio-oil yield. The zeolite catalyst could effectively perform deoxygenation and denitrogenation while simultaneously increasing aromatic compounds in bio-oil. In the same study, pyrolysis of *Spirulina platensis* resulted in a bio-oil yield of 49.9%. Bio-oils from pyrolysis of *Nannochloropsis* sp. and *Spirulina platensis* were composed of aliphatic, cyclic, aromatic hydrocarbons and oxygenated and nitrogenous compounds [79]. Nevertheless, the study demonstrated that both marine and freshwater microalgae strains could be pyrolyzed successfully to produce approximately 50% bio-oil (Table 4), which, if not further upgraded, could be used as a fuel oil for power generation. The bio-oil yield from the pyrolysis of *Scenedesmus obliquus* biomass at 500 °C in the absence and presence of a catalyst was 46.3 and 17%, respectively. The presence of a catalyst reduced bio-oil yield and increased gas fraction. The calorific value of bio-oils derived via non-catalytic and catalytic processes was 36.9 and 39 MJ/kg, respectively [80]. The catalyst could lead to deoxygenation of the bio-oil, improving its higher heating value (HHV). In another study, microalgae have been reported as a co-feedstock in the pyrolysis process for bio-oil production [81]. Pyrolysis of *Nannochloropsis* sp. biomass and bamboo waste

mixture at a ratio of 1:1 resulted in a bio-oil yield of 66.63%. In contrast, a blend of 50% *Chlorella vulgaris* and 50% waste glycerol resulted in a bio-oil yield of 59% with an HHV of 24.9 MJ/kg [82].

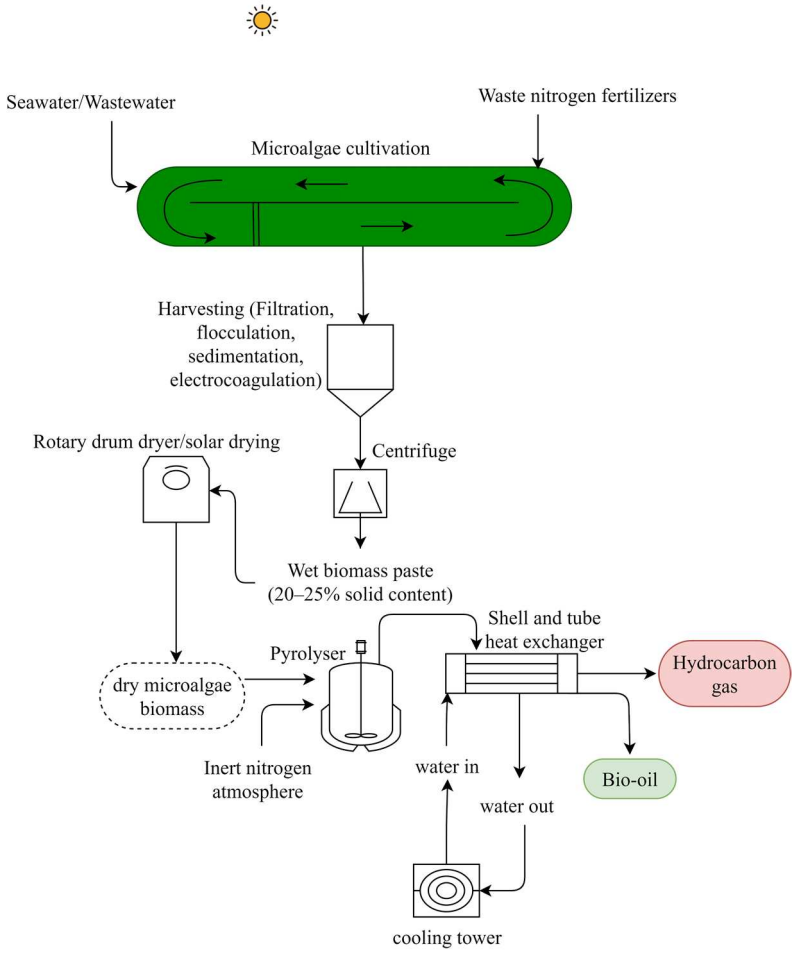



**Figure 4.** Process flow diagram for Bio-oil production using pyrolysis process from microalgae.

**Table 4.** Pyrolysis oil production from various microalgae strains.

| Microalgae Feedstock | Pyrolysis Conditions | Bio-Oil Yield (%) | Reference |
|---|---|---|---|
| *Spirulina platensis* | 400–600 °C, molten salt mixture | 58–60 | [83] |
| *Chlorella sorokiniana* | 516 °C, 17 min. | 32.3 | [84] |
| *Chlorella vulgaris* and municipal sewage sludge (MSS) | 520 °C, argon gas | 45.6 | [85] |
| *Nannochloropsis* sp. | 15 mg sample, heated from 35 to 800 °C | 52.2 | [86] |
| *Desmodesmus* sp | 350–750 °C, | 41.9 | [87] |

### *2.5. Bio-Jet Fuel*

Fossil-based jet fuel is the upgraded kerosene obtained from the distillation of fossil crude oil; it is also known as aviation fuel [88,89]. Jet fuel is a mixture of paraffin, naphthenes, olefins, and aromatics and contains 6 to 16 carbon atoms in its chain structure. Generally, it is composed of 40% iso-paraffin and 20% paraffin, the remaining being naphthenes and aromatics [90]. Jet A and Jet A-1 fuels are used in the USA and are compatible with presently used commercial turbines [91]. Jet B, a mixture of kerosene and gasoline, is used in very cold climates, e.g., Alaska and certain cold regions in Canada [92]. Another important feature of jet fuel is that the energy density of the fuel needs to be high, i.e., the

H/C ratio must be near 2, and the O/C ratio needs to be low with the minimum presence of oxygen [93]. The presence of oxygen in the jet fuel could lead to the formation of water molecules, decreasing its energy density [94].

Currently, iso-paraffinic-rich bio-jet fuel is produced from hydrotreated esters and fatty acids (HEFA) using a catalytic process [95]. The potential pathway of bio-jet production from microalgae biomass is shown in Figure 5. However, in the present scenario, bio-jet fuel from microalgae has not been commercialized due to a high processing cost [96]. Therefore, a recent study explored the potential of *Schizocytrium* sp. biomass as feedstock for producing bio-jet fuel and high-value polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) and docosapentaenoic acid [97]. 50% lipid was extracted using a 1:1 ethanol and hexane mixture from heterotrophically cultivated *Schizocytrium* sp. The extracted lipids were then converted to fatty acid ethyl esters (FAEE). Later, the saturated FAEEs were separated from polyunsaturated FAEEs using a short path distillation unit. From the saturated FAEEs, catalytic deoxygenation yielded 75% liquid paraffin, which was later converted to iso-paraffin (i.e., $C_8$–$C_{16}$ compounds) by catalytic hydrocracking. Further fractional distillation of iso-paraffin resulted in 20 wt% bio-jet fuel that satisfied the ASTM D7566 fuel specifications. The bio-jet fuel was produced from microalgae *Schizocytrium* sp., wherein the separation of high-value polyunsaturated (FAEE) could offset the cost of bio-jet fuel synthesis from a lipid-rich microalgae feedstock [97]. In another study, in a fixed bed reactor, algal triglycerides were converted to n-alkanes using a 3% palladium/carbon catalyst at 350 °C and 55 bar of hydrogen pressure. Around 95% of algal triglyceride was deoxygenated and converted to n-alkanes; later, bio-jet fuel from n-alkanes was synthesized by hydrocracking using 0.5% Pt/USY-zeolite catalyst. Although the successful conversion of algal triglycerides to bio-jet fuel was feasible, there were major losses of compounds such as $C_8$ Naptha; hence, the overall bio-jet fuel yield was reduced, and it was difficult to achieve the desired low-temperature jet fuel specification [95]. Biojet fuel produced from various microalgal feedstocks and their corresponding yields are shown in Table 5.

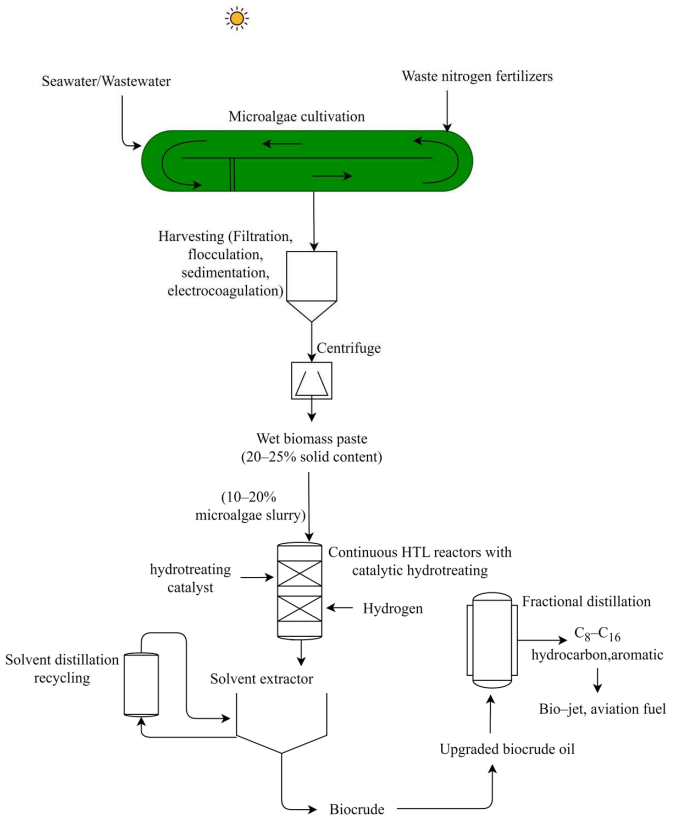



**Figure 5.** Process flow diagram for Biojet fuel production from microalgae.

**Table 5.** Biojet fuel production from various microalgae strains and lipids.

| Microalgae Feedstock | Processing Parameters | Biojet Fuel Yield (%) | Reference |
|---|---|---|---|
| *Chlorella* sp. lipids | Hydrodeoxygenation, hydrocracking, and hydroisomerization with hydrogen gas at 410 °C and 50 bar pressure in the presence of NiO, $MoO_3$/H-ZSM-5 catalyst | 76 | [98] |
| *Botryococcus braunii* oil | Hydrocracking at 400 °C and 200 bar pressure with hydrogen gas in the presence of a cobalt-molybdenum catalyst. | 15 | [99] |
| *Chlorella* sp. | Continuous hydrothermal liquefaction and hydroprocessing at 350 °C, residence time of 1 to 5 min, hydroprocessing in the presence of nickel and cobalt molybdenum catalyst at 405 °C. | 40 | [100] |
| Microalga sp. lipids | Hydrotreated at 260 °C and 40 bar in the presence of Ni/HBeta and HZSM-5 catalyst. | 78 | [101] |
| *Nannochloropsis* sp. lipids | Hydroprocessed at 375 °C, 5 bar in the presence of cobalt molybdenum aluminum oxide catalyst | 35 | [102] |

### 2.6. Biomethane

Biomethane is a product of anaerobic digestion (AD), where organic-rich waste streams, either liquid or semi-solid, are anaerobically digested by methanogenic bacteria [103]. The product of AD is commonly known as biogas, which comprises $CH_4$ (55–75%) and $CO_2$ (20–40%) [104]. A trivial amount of $H_2$, $H_2S$, $N_2$, and water vapor is observed in biogas as impurities [105]. The typical methane yield varies in the range of 200–400 L $kg^{-1}$ algal biomass or 24–60 L $g^{-1}$ volatile solids (VS), depending on the strains and the operational conditions [106]. Although the rate of methane production is low, bio-methanation is a robust process. AD primarily involves the following four processes: hydrolysis; acidogenesis; acetogenesis; and methanogenesis [107]. Methanogen bacteria come under the archaea family, which includes *Methanobacterium* sp., *Methanospirillum* sp., *Methanococcus* sp., and *Methanosarcina* sp. [108]. Like other carbonaceous feedstocks, microalgae biomass can be used as a substrate in bio-methanation (Figure 6). Nevertheless, methane production using microalgae as a substrate in AD is influenced by many factors, such as the strength of cell-wall of the algal strain used, C/N ratio, the salt concentration in media, cultivation conditions of microalgae, and the operating parameters of AD [104]. Cellular membrane degradation is a limiting step when algal biomass is used as a feedstock in biogas production, and the digestibility of the wall depends on algal species. Table 6 shows pretreatment steps typically used for disintegrating microalgal cell walls, along with biomethane yields from various microalgal strains. *Dunaliella salina*, *Chlamydomonas reinhardtii*, and *Euglena gracilis* had better digestibility in AD than *Scenedesmus obliquus* and *Chlorella kessleri* because of differences in their cell wall structures [104]. Typically, cyanobacteria do not have rigid cell walls; hence, cyanobacteria are preferable over microalgae [109]. The high content of nitrogen in feedstock's could generate $NH_4^+$, which showed an inhibitory effect on methane production in AD [110]. Therefore, it was recommended to maintain a high C/N ratio (25–30) [109]. However, the C/N ratio in algal biomass is reported as 5.3–10.2 [111]. Therefore, co-digestion of microalgae (or de-oiled biomass) with carbonaceous biomass with a high C/N ratio could be promising for methane production. Hu et al. (2021) utilized a mixed microalgal consortium, dominated by *Tribonema* sp., as a co-substrate with pig manure, in the ratio of 0.5:0.5 (VS basis) in AD. The performance analysis revealed that adding microalgae as a co-substrate enhanced methane yield and energy recovery by 27.4% and 81%, respectively [112]. In contrast, the C/N ratio above 35 is unsuitable for AD because it leads to low methane yield [113]. Since marine microalgae usually have high salt content, it could affect biomethane production, as methanogens require a low amount of sodium. The optimum range of sodium concentration is 100–350 mg $L^{-1}$ to maintain ideal osmotic pressure for methanogenic bacteria [104].

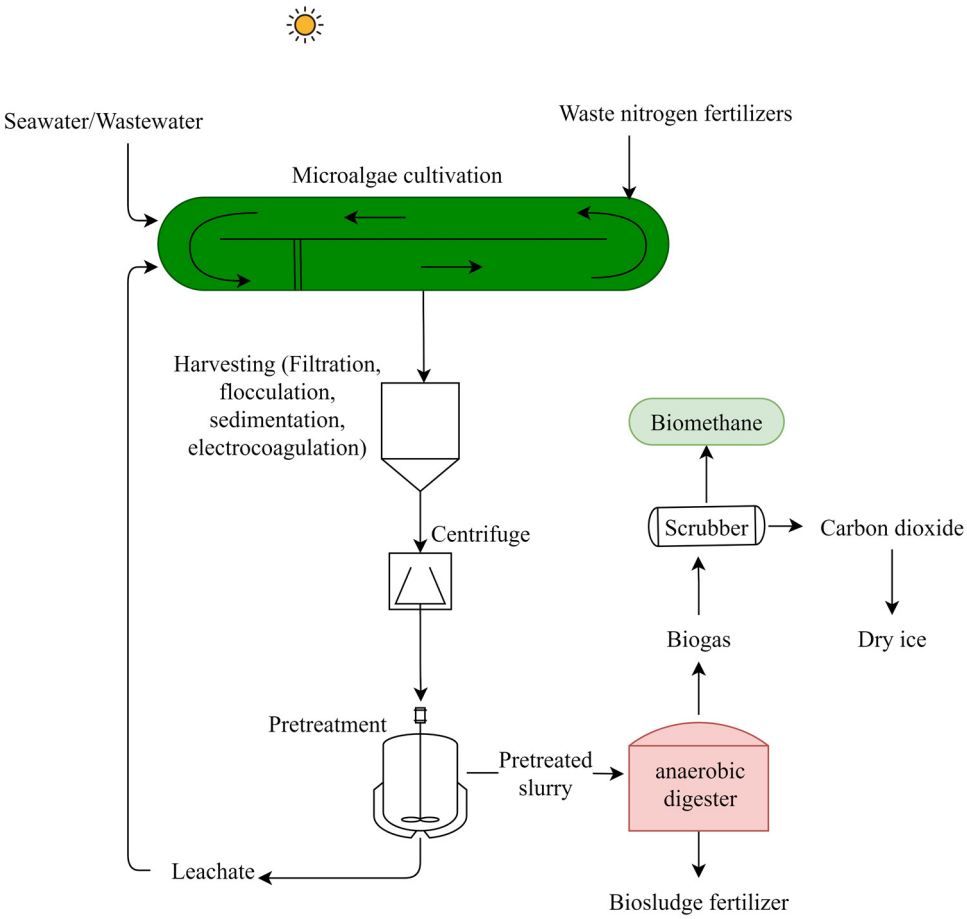



**Figure 6.** Process flow diagram for biomethane production from microalgae.

Methane yield is significantly affected by the composition of microalgal biomass, which depends on the cultivation conditions. For example, when microalgae are under nutrient-stress conditions, they produce more lipids or carbohydrates than protein. A higher concentration of lipids in the feedstock of AD is desirable for methane production [114]. Nevertheless, a proper balance between lipid and protein content in the substrate is required to maintain the alkalinity of the broth medium. Apart from the above process parameters, pH, temperature, and the organic loading rate could influence methane production in AD [115]. For instance, the optimum pH for methanogenesis in AD is 6–8; however, the suitable pH for the first three steps (hydrolysis, acidogenesis, and acetogenesis) is 4.5–6 [113]. Therefore, automatic process monitoring is advisable for the maximum efficiency of AD.

**Table 6.** Biomethane production from various microalgae strains.

| Microalgae Feedstock | Pretreatment Process Parameters | Biomethanation Process Conditions | Biomethane Yield (mL/g VS) | Reference |
|---|---|---|---|---|
| *Chlorella pyrenoidosa* | Hydrolysis by hydrothermal pretreatment using a parabolic solar thermal system | The feed flow rate of 40 L/h, retention time 30 min and mass fraction 1% | 348 | [116] |
| *Chlorella vulgaris* | Working vol 2.8 L, pretreated at 85, 55 °C, total hydraulic retention time–6 days. | Mesophilic anaerobic digestion @ 35 °C | 239.3 | [117] |
| *Chlamydomonas reinhardtii* CC-1690 | Replete Nitrogen and low Nitrogen | 38 °C, hydraulic retention time 20 days, organic loading rate 4 g VS/d | 464 | [118] |
| *Chlorella* sp. | Pretreated at 60–80 °C, for 5 to 10 min | Mesophilic temperature 35 °C, for 46 d | 252 | [119] |
| *Scenedesmus* sp. | Nitrogen and Phosphorus depleted | 37 °C | 320 | [120] |

### 2.7. Biohydrogen

Hydrogen is considered the fuel of the future because of its non-polluting nature and high energy density (142 kJ/g) [121]. Hydrogen consumption has expanded globally and is expected to provide 10% of the energy market by 2025 [122]. Biological hydrogen production using microalgae can be done either by bio-photolysis, where microalgae act as a biocatalyst, or fermentation, where algal biomass is utilized as feedstocks [109]. Bio-photolysis can be categorized as direct and indirect, whereas fermentation can be classified as photo- and dark fermentation [123].

Water is split into $H_2$ and $O_2$ by green microalgae and cyanobacteria through bio-photolysis. In direct photolysis, hydrogenase in green microalgae catalyzed the reaction of $H_2$ synthesis. In contrast, nitrogenase in blue-green algae (cyanobacteria) facilitates $H_2$ production via nitrogen fixation [124]. Several microalgae, such as *Chlamydomonas reinhardtii*, *Platymonas subcordiformis*, *Scenedesmus obliquus*, *Chlorococcum littorale*, *Chlorella fusca*, etc., have an Fe-Hydrogenase enzyme, and these strains can participate in direct bio-photolysis [125,126]. Nevertheless, the hydrogenase enzyme, the primary precursor of $H_2$ synthesis, is highly sensitive to oxygen, which inhibits the production process in direct bio-photolysis [123]. In contrast, $H_2$ production via indirect bio-photolysis occurs in a heterocyst that shields oxygen-sensitive nitrogenase against $O_2$ contact by sulfur deprivation [127]. Although it was first stated as indirect bio-photolysis, it is, in fact, a direct bio-photolysis respiratory consumption of the $O_2$ released [127]. Cyanobacteria associated with indirect bio-photolysis for biohydrogen production are *Anabaena* sp., *Synechococcus* sp., *Oscillatoria* sp., *Gloebacter* sp., etc. [123]. However, it was noticed that sulfur depletion or repletion in indirect bio-photolysis led to lower hydrogen yield [128]. Owing to its many technological deficiencies, bio-photolysis is not yet viable for biohydrogen production [129].

To overcome the low efficiency of bio-photolysis, researchers have explored the use of algal biomass (including the de-oiled biomass) as a feedstock (Figure 7, Table 7) in microbial fermentation for $H_2$ production [130]. Although photo-fermentation cannot directly convert algal biomass into $H_2$, it can use organic acids such as acetic-, butyric-, succinic acid, by-products of dark fermentation (DF), etc., to produce $H_2$ and $CO_2$ under anaerobic conditions [123]. The photosynthetic bacteria involved in photo-fermentation are *Rhodobater* sp., *Rhodospirillum* sp., *Rhodopseudomonas* sp., *Halobacterium* sp., *Chromatium* sp., etc. [131]. For example, the effluent of DF (*Spirulina platensis* was used as substrate in DF) was utilized as a feedstock in photo-fermentation to produce 1510 mL $L^{-1}$ $H_2$ using an inoculum volume of 20% [132].

Despite having a high biohydrogen yield, photofermentation has several drawbacks, such as low solar conversion efficiency, difficulties in maintaining anaerobicity in substantial areas of a photobioreactor, and high energy demand for biocatalysts [133]. On the other hand, DF is advantageous because it does not require light sources—unlike bio-photolysis and photo-fermentation—can utilize diverse feedstocks, and is scalable [121,134]. In DF, similarly to ethanol fermentation, algal carbohydrates are used as the substrate to produce $H_2$ and $CO_2$ as gaseous products and volatile fatty acids as liquid products using a diverse group of anaerobic microbes. The microbes, which are abundant in nature, can be either mesophilic (25–45 °C) or thermophilic (45–60 °C) [135]. Metabolism of $H_2$ production is thermodynamically more favorable in thermophiles than mesophiles [136]. Similarly, based on the $O_2$ tolerance, these bacteria can be further classified as obligate and facultative. Obligate (strict) anaerobic bacteria cannot survive in aerobic conditions. Therefore, $O_2$ concentration in the growth medium should be below 0.02–0.04% [123]. The abundant strict anaerobes are *Clostridium* sp., *Thermoanaerobacterium* sp., and *Ethanoligenes* sp. [137]. In contrast, facultative bacteria can sustain some aerobic conditions and produce $H_2$ in anaerobic conditions. The most commonly studied facultative anaerobes are *Enterobacter* sp., *Klebsiella* sp., and *Escherichia coli*, Bacillus sp. [138,139]. Recently, the mixed consortium has gained attention from researchers because of its applications in a diverse range of feedstocks, ease of control, and low operational cost [121].

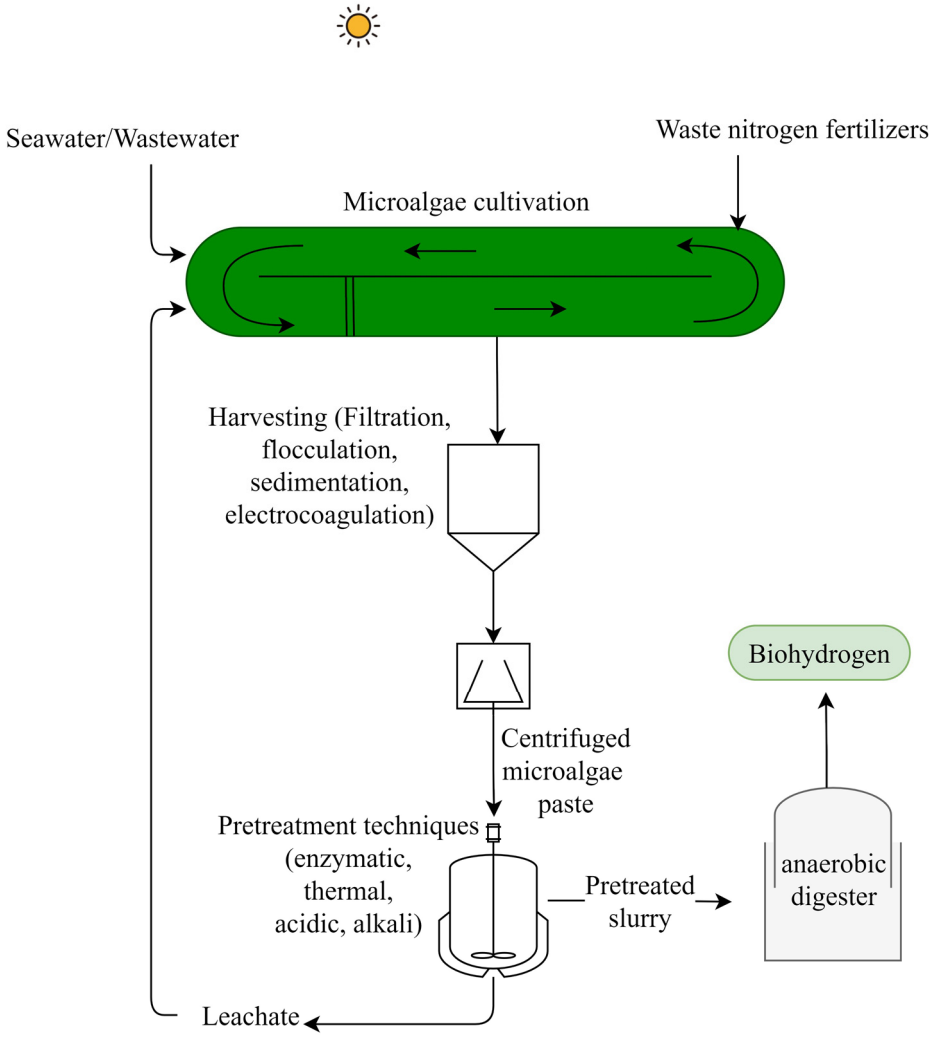



**Figure 7.** Process flow diagram for biohydrogen production from microalgae.

**Table 7.** Biohydrogen production from various microalgae strains.

| Microalgae Feedstock | Pretreatment Process Parameters | Fermentation Process Conditions | Biohydrogen Yield | Reference |
|---|---|---|---|---|
| *Chlorella vulgaris* | Enzymatic, 60 h | pH 7.4, 3 N KOH, 35 °C, 150 rpm | 43 mL $g^{-1}$ | [140] |
| Mixed microalgae consortia | 121 °C, autoclave | $N_2$ sparging, 220 rpm, 30 °C, 8h | 56.8 mL $g^{-1}$ VS | [141] |
| *Tetraspora* sp. | 140 rpm, 36 °C, 7.2 pH | 140 rpm, 36 °C, 29 µmol photons $m^{-1}$ $s^{-1}$, 18 h | 512 mL $L^{-1}$ | [142] |
| *Chlorella* sp. | 1.5% hydrochloric acid, 121 °C, 20 min | pH 7, 37 °C, 9 g reducing sugar/L | 1276 mL $L^{-1}$ | [143] |
| *Chlamydomonas* sp. | 3 h dark adaptation | 25 °C, 4.5–9 h. | 8.73 L $kg^{-1}$ | [144] |

Since the production of $H_2$ from microalgae is directly proportional to carbohydrate content, Singh et al. (2019) first maximized the carbohydrate content in *Scenedesmus obliquus* by optimizing process parameters and then utilized the de-fatted algal biomass for dark fermentative $H_2$ production using mixed acidogenic consortia [145]. The maximum carbohydrate content in the algal strain grown under optimized conditions (mixotrophic) was 55.40%, and subsequently, the highest $H_2$ production obtained was 428.5 mL $g^{-1}$ of carbohydrate. Furthermore, microalgae biomass could be used as a co-substrate in DF to

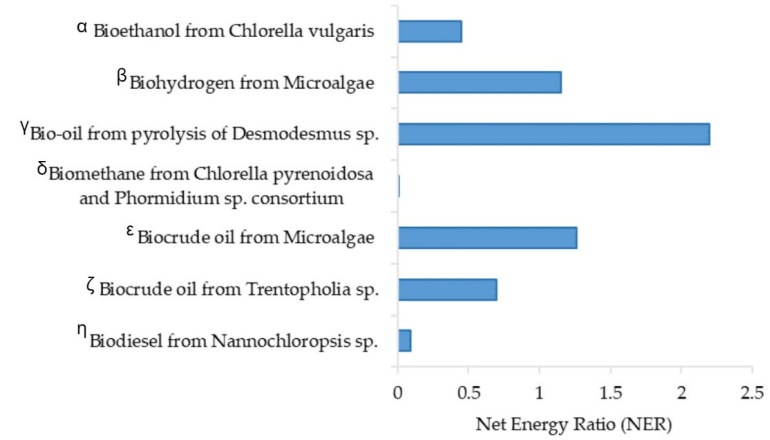
enhance production. Yin et al. (2021) investigated the co-fermentation of algae biomass and sewage sludge and achieved the $H_2$ yield of 14.8 mL $g^{-1}$ VS, which was obtained more than two-fold from mono-fermentation [146]. In addition, DF has great potential for circular bioeconomy. For example, the effluent of DF, comprised of volatile fatty acids, can be utilized as a substrate for mixotrophic microalgae cultivation [147]. Similarly, the anaerobic sludge generated in the DF reactor is a promising source of extracellular polymeric substances (EPS), which could be applied to microalgae harvesting through bio-flocculation [148].

## 3. Comparative Net Energy Ratio and Carbon Dioxide Emission from Different Microalgal Biofuels

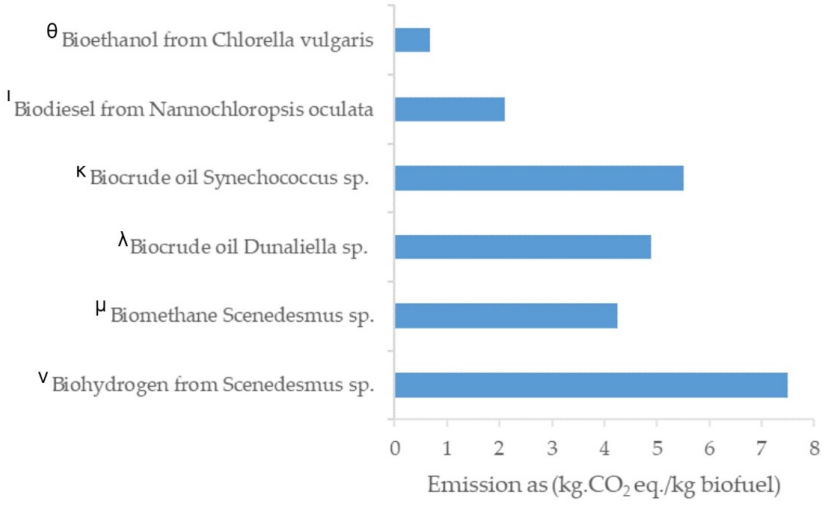
This section gives a general overview of energy efficiency and carbon emission from different microalgal biofuels, although the net energy ratio and carbon dioxide emission for any type of microalgal fuel will vary based on microalgal strains, cultivation conditions, downstream processing, etc. The net energy ratio (NER) is defined as the ratio of the total energy produced over the required processing energy [149]. The NER for biodiesel produced from *Nannochloropsis* sp. using a conventional oil extraction process followed by a transesterification process was less than 1 (Figure 8a); energy-intensive unit operations such as drying and cell disruption could have reduced the NER of biodiesel [150]. A study reported a NER value of <1 for HTL biocrude of the microalgae consortium, with the predominance of strain *Trentepholia* sp.; however, the reported biocrude yield was a mere 18%, with microalgae solid loading of 4%, which could have lowered the NER value [151]. Another study reported a higher NER for biocrude oil produced from a large-scale HTL system from a model microalgal biomass (Figure 8a); it also reported that NER for biocrude oil produced from various microalgae could range from 1 to 1.2 [152]. Still, the NER could vary based on the selected strain, cultivation conditions, and energy requirement of the HTL process. The biogas obtained from the consortium of *Chlorella pyrenoidosa* and *Phormidium* sp. grown in wastewater had the lowest NER among the selected microalgal biofuels (Figure 8a) [153]. Any further scrubbing of biogas for further purification could have reduced net energy ratios further [153]. Furthermore, the NER of biodiesel, biomethane, and biocrude has been reported to be below 1, as shown in Figure 8a, rendering these microalgal biofuels unfavorable for commercial applications. Another life cycle assessment (LCA) study reported a NER > 1 for microalgal hydrogen production, wherein supercritical water gasification of microalgae biomass was assumed instead of conventional fermentation techniques [152].

In another study, hydrogen and biomethane were produced from *Scenedesmus* sp. using reactive flash volatilization (RFV); the climate change impact of producing hydrogen and biomethane was estimated using the LCA model (Figure 8b). Biomethane production was reported as environmentally more friendly than hydrogen production. The higher positive impact from electricity requirements, nutrients, and the scrubbing operation involved in hydrogen production could lead to a higher environmental impact than biomethane [154]. A recent study compares the climate change impact of bio-crude oils from five microalgae strains (Figure 8b). Among the studied strains, the non-settling *Synechococcus* sp. had a high climate impact due to harvesting energy requirement; conversely, *Dunaliella* sp. had the lowest climate impact potential among selected strains due to low nutrient, lower cultivation, and harvesting energy requirements [155]. The climate impact of microalgal biocrude oil is still lower than the corresponding value for microalgal biohydrogen (Figure 8b); this could be due to the supercritical gasification technique [152]. A laboratory-grown *Desmodesmus* sp. was pyrolyzed, and the NER value of the pyrolytic oil was higher compared to the NER values of other biofuels (Figure 8a). Higher NER of pyrolytic oil compared to other strains could be due to microalgae cultivation in anaerobically digested wastewater that could have offset the cultivation energy; the major energy input contribution was from the harvesting unit operation. Additionally, high HHV of pyrolytic oil could have improved the NER of the pyrolysis process. Furthermore, the pyrolysis of 1 kg *Desmodesmus* sp. at

600 °C was reported to have a high adverse climate impact value of 2423 kg $CO_2$ eq./kg, which was the highest among the other selected biofuels (not shown in Figure 8b); generation of $CO_2$, nitrogenous gases, carbon monoxide, and methane during the pyrolysis process were linked to negative climate impact [156]. Although the NER was high for bio-oil production from *Desmodesmus* sp., the climate impact was also significantly higher, having a negative impact on the environment. In another study, for producing microalgal methyl ester (i.e., biodiesel), the production had an impact value of 2.06 kg $CO_2$ eq./kg biodiesel, the lowest contributor of $CO_2$ emission impact among the selected microalgal biofuels (Figure 8b). An LCA study was conducted for biodiesel production using *Nannochloropsis oculata* biomass by assuming the cultivation size of 80 ha pond and cellular lipid content of 46%; the GHG emission was estimated as 2.1 kg $CO_2$ eq./kg biodiesel [157]. Lower climate impact values for biodiesel and bioethanol than other selected biofuels could be due to mild processing conditions and their low power requirements [158]. The NER value and $CO_2$ emissions, as shown in Figure 8a,b, favor HTL and pyrolysis as promising technologies that could be practically feasible in the near future. Strain selection, optimization of harvesting techniques, and processing of wet biomass would determine the overall practical feasibility of microalgal biofuels produced using HTL and pyrolytic technologies.




(**a**)




(**b**)

**Figure 8.** (**a**) Net energy ratio of biofuels produced from microalgal biomass. $^{\alpha}$ [150]; $^{\beta}$ [152]; $^{\gamma}$ [156]; $^{\delta}$ [153]; $^{\varepsilon}$ [152]; $^{\zeta}$ [151]]; $^{\eta}$ [150]. (**b**) Climate change impact as (kg.$CO_2$.eq./kg) of biofuels produced from microalgal biomass. $^{\theta}$[158] $^{\text{calculated}}$; $^{\iota}$ [157]; $^{\kappa}$ [155]; $^{\lambda}$ [155]; $^{\mu}$ [154]; $^{\nu}$ [154].

## 4. Cost Analysis of Various Biofuels Produced from the Microalgal Feedstock

The production cost based on earlier reported techno-economic analysis (TEA) models for various microalgal biofuels, described in this study, is shown in Table 8. Furthermore, the cost breakdown for the reported TEA studies on microalgal biofuels has been analyzed based on the ratio of capital expenditure (Capex) to operating expense (Opex) for each fuel type. Later, in the following section, for each selected microalgal biofuel, a short cost description is provided by comparing two studies on that biofuel.

### *4.1. Microalgal Biodiesel*

In 2011, it was reported that biodiesel production from microalgae could reach 1 US$ per unit of fuel produced (Table 8); additional improvements in microalgae cultivation in open raceway ponds could push biodiesel produced from microalgae more toward commercialization. Another study in 2022 reported biodiesel cost as 0.9 US$/kg (Table 8), which is competitive with the existing conventional fossil diesel fuel current price of 1.2 US$L$^{-1}$ in the United States. Furthermore, as shown in Figure 9, the major contributor towards Capex was the procurement of biodiesel plant machinery and accessories, whereas the major cost in Opex was from fixed charges that included taxes, interest, plant overhead cost, and insurance.

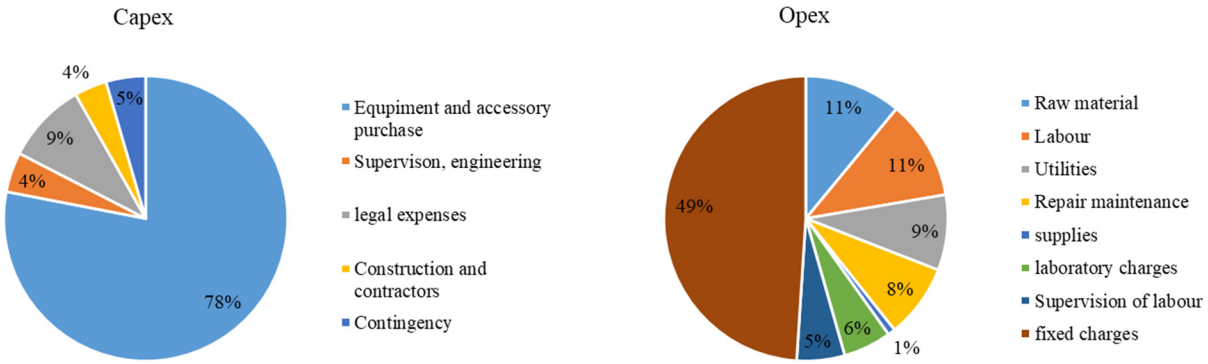



**Figure 9.** Cost distribution for producing 5.4 m$^3$/d biodiesel from microalgae biomass (82.09% of the total cost was utilized as Capex and 17.90% as Opex) [159].

Other than fixed charges, raw materials for transesterification reaction using calcium oxide catalyst and methanol, with required labor, were the other major contributors in OPEX for microalgal biodiesel production [159]. The biodiesel production cost in both studies (Table 8) could be lowered by using ORPs for large-scale microalgae cultivation. However, further cost reduction in biodiesel price could be achieved by efficient lipid extraction, thereby reducing the cost of catalysts, and efficient solvent recycling systems.

### *4.2. Microalgal Biojet Fuel*

The reported cost of biojet fuel from microalgae is on the higher side when compared with other reported biofuels (Table 8). The production facility, microalgae ponds, and harvesters were the major drivers in the Capex of microalgal biojet fuel; other cost contributors were solvent extractors, hydrocracking, and hydrotreating equipment.

Similar to biodiesel Opex, the major contributors to Opex of biojet fuel production were annual operating costs (Figure 10), i.e., insurance, depreciation followed by facility overhead, and maintenance. Harvesting was another major cost contributing unit operation in annual operating cost, followed by raw material, wherein 90% of water and nutrients were recycled after the harvesting process; still, the water was reported to be a major contributor (10.11%) to raw materials [160]. As shown in Table 8, the cost of biojet fuel could range between 5.89 to 8.45 US$/L; in both the reported studies harvesting unit operation was the major cost input for producing microalgal biojet fuel. It was reported that if the harvesting cost could be reduced, then the cost per barrel of biojet fuel could

be lowered by 70%—thus improving the economic viability of biojet fuel production from microalgae biomass.

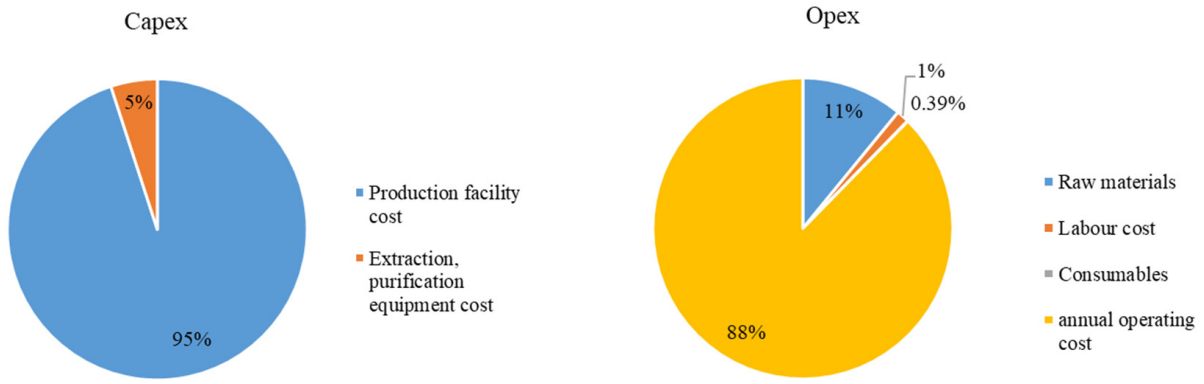



**Figure 10.** Cost distribution for producing per barrel of biojet fuel from microalgae biomass (79.4% of the total cost was utilized as Capex and 20.5% as Opex) [161].

**Table 8.** A comparative economic analysis of various biofuels produced from microalgae biomass.

| Biofuel from Microalgae | Microalgal Strain | Strain Habitat | TEA Model | Production Cost US$ per Unit Fuel | Commercial Fuel Cost US$ per Unit Fuel | Reference |
|---|---|---|---|---|---|---|
| Biodiesel | *Nannochloropsis salina* | Marine | Simulation model using (ASPEN plus V.12) for 5.4 $m^3$/day biodiesel, cultivation area 153 ha. | 0.77 $L^{-1}$ | 1.15 $L^{-1}$ | [159] |
| Biodiesel | *Chlorella vulgaris* | Freshwater | SAFEER model, 5 to 50 ha scale ORP cultivation of *Chlorella vulgaris* | 0.8–3.5 $L^{-1}$ | 1.15 $L^{-1}$ | [162,163] |
| Bioethanol | *Chlorella vulgaris* | Freshwater | Model for producing 24.9 $m^3$ $ha^{-1}$ $yr^{-1}$ | 19.45 $gal^{-1}$ | 2.718 $gal^{-1}$ | [164] |
| Bioethanol | Microalgae | - | Algenol LLC, photobioreactors, 8000 gal/acre/yr | 1.3 $gal^{-1}$ | 2.718 $gal^{-1}$ | [165,166] |
| Biohydrogen | *Chlamydomonas* sp. | Marine | Photobioreactor | 0.57–13.53 $kg^{-1}$ | 2–8 $kg^{-1}$ | [167] |
| Biohydrogen | Model microalgae | - | Photobioreactor | 2.13 $kg^{-1}$ | 2–8 $kg^{-1}$ | [168,169] |
| Biomethane | *Cyanothecae* BG0011 | Marine | Modeled at 80,300 kg/h/yr, with biomethane purification using ASPEN V 8.8 | 0.55 $m^{-3}$ or 14.8/MMbtu | 0.25 to 2.7 $m^{-3}$ | [170,171] |
| Biomethane | *Spirulina* sp. | Freshwater | - | 0.3 $m^{-3}$ | 0.25 to 2.7 $m^{-3}$ | [172] |
| Biocrude oil | Microalgae powder | | ASPEN plus simulation | 2.2 $L^{-1}$ | 0.48–0.53 $L^{-1}$ | [173,174] |
| Biocrude oil | *Lipid-extracted algae—Nannochloropsis salina* | marine | ASPEN plus for HTL processes | 0.7 $L^{-1}$ | 0.48–0.53 $L^{-1}$ | [175] |
| Pyrolysis oil | *Chlorella vulgaris* | Freshwater | A model to process 2000 metric tonnes of biomass to produce 21.4 million gallons of pyrolysis oil | 1.48–1.8 $L^{-1}$ | 0.71 $L^{-1}$ | [176,177] |
| Pyrolysis oil | Microalga | Centrate wastewater | - | 0.58 $L^{-1}$ | 0.71 $L^{-1}$ | [178] |
| Biojet fuel | *Nannochloropsis* sp. | Marine | - | 5.89 $L^{-1}$ | 0.9 $L^{-1}$ | [161,179] |
| Biojet fuel | *Nannochloropsis* sp. | Marine | - | 8.45 $L^{-1}$ | 0.9 $L^{-1}$ | [160] |

### *4.3. Microalgal Biohydrogen*

The cost to produce biohydrogen was reported to be higher than the production cost for biomethane (Table 8). In direct biophotolysis, microalgal biohydrogen production

has low photochemical efficiency, and oxygen accumulation adversely affects hydrogen production during the process. Furthermore, indirect biophotolysis for biohydrogen by microalgae requires fermentation. In the indirect biophotolysis process, an appropriate strain—having a lower hydrogenase sensitivity towards oxygen could be cultivated in carbohydrate-rich wastewater to reduce the cost of biohydrogen production [180]. A TEA study considered a 110,000 $m^2$ ORP in Arizona with a pond depth of 10 cm for obtaining a 0.2 g $L^{-1}$ biomass density; the harvested biomass was utilized for biohydrogen productivity of 300 kg $d^{-1}$ [167]. As shown in Figure 11, the major contribution towards Capex comes from engineering and construction, which includes reactor design and construction, hydrogen compression, hydrogen transfer pipeline design and construction, and high-pressure hydrogen storage, followed by the construction of algal ponds.

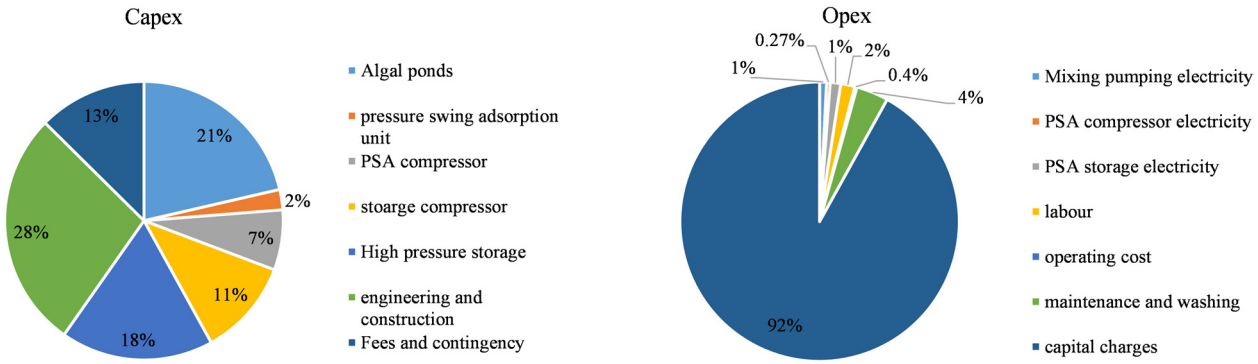



**Figure 11.** Cost distribution of 300 kg/d biohydrogen production from microalgae biomass (77.6% of the total cost was utilized as Capex and 22.3% as Opex) [167].

In Opex, other than capital-related charges such as taxes, insurance, and overhead expenses, the major cost contribution was from maintenance and washing costs due to periodic pond washing and onsite maintenance of plant equipment. Biohydrogen cost can range from 0.5 to 13.7 US$/kg. The cost of biohydrogen could be reduced by lowering the cultivation reactor cost and using pressurized hydrogen pipelines instead of hydrogen storage systems. Additionally, metabolic and genetic engineering techniques need to be applied to establish a sustainable biohydrogen production system.

### *4.4. Microalgal Biomethane*

The cost of biomethane from microalgal biomass could be reduced by incorporating fermentation process-derived $CO_2$ in algal ponds, high-rate anaerobic digesters, and pre-concentrating microalgae biomass to 2 to 6% using filtration processes [181]. A TEA study was conducted to determine biomethane production cost from *Cyanothecae* sp. biomass (Figure 12) using a mesophilic anaerobic digestion process. For the biomethane production plant, the major contribution to Capex came from the anaerobic digester cost [170].

In Opex, the major contributor was the cost of raw material, i.e., the algal biomass (Figure 12). Although the carbon credit was reported to be 10 US$/tonne, still the reduction in algal biomass raw material cost was minimal. Another way of reducing feedstock production cost was proposed by increasing biomass productivity by enriching the air with $CO_2$ (1% *v*/*v*) and introducing it in the raceway pond. As shown in Table 8, the reported cost of biomethane produced from *Spirulina* sp. (0.3 \$/$m^3$) was comparatively lower than that of *Cyanothece* sp. (0.55 \$/$m^3$). While a biorefinery approach was adopted to utilize *Spirulina* biomass digestate as a biofertilizer, the cost of biomethane production was lower than that of *Cyanothece* sp.

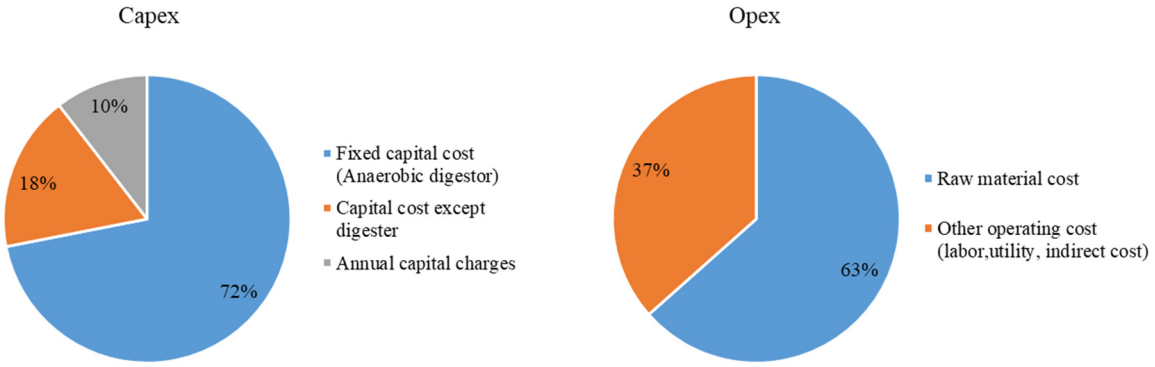



**Figure 12.** Cost distribution for Biomethane production from microalgae biomass (56.9% of the total cost was utilized as Capex and 43% as Opex) [170].

### *4.5. Microalgal Biocrude Oil*

Biocrude oil produced using hydrothermal liquefaction (HTL) has been reported to be the lowest in cost among the selected microalgal liquid biofuels for this study. HTL of biocrude oil has been extensively studied, as it is considered one of the most promising techniques due to the exclusion of drying and oil extraction steps. Over the past decade, microalgal HTL with integrated biorefining recycling systems coupled with simultaneous catalytic hydrotreating for improving the biocrude quality has shown the potential to reduce biocrude production cost [70]. As shown in Figure 13, fixed capital investment was a major contributor to Capex. An earlier TEA study distributed the fixed capital components to working capital requirement, interest, local taxes, charges, and depreciation [173].

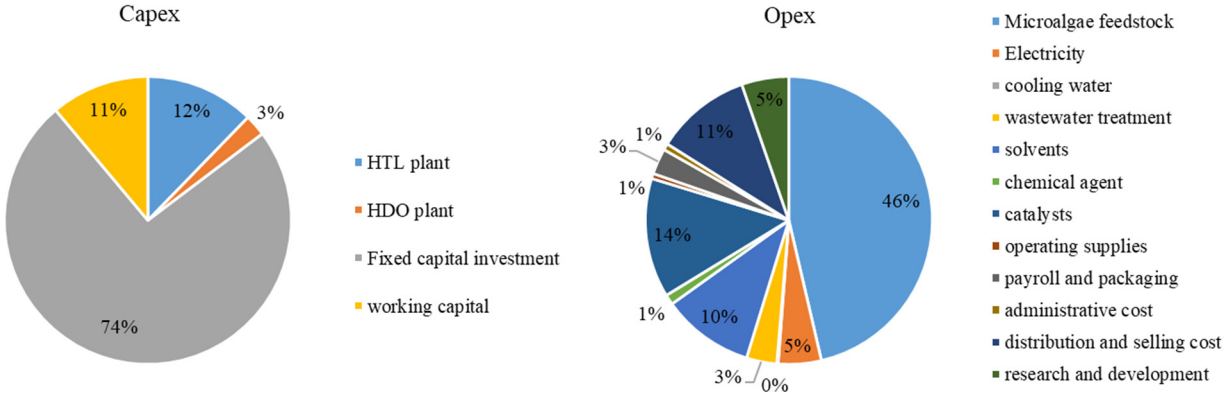



**Figure 13.** Cost distribution for 83.81 tonnes of day$^{-1}$ biocrude from microalgae biomass (42.3% of the total cost was utilized as Capex and 57.6% as Opex) [173].

The remaining capital cost came from developing the HTL and hydrodeoxygenation (HDO) plants. The major contributor towards Opex was the procurement of microalgae powder for HTL from an external source. Although the microalgae biomass was neither cultivated nor harvested in the reported study, the cost of procuring it as a direct raw material contributed to 39% of the operational cost. Besides microalgae biomass, the solvents used for extracting biocrude were reported to have made a major contribution to Opex. As shown in Table 8, the cost of biocrude oil produced from lipid-extracted microalgae (LEA) was comparatively much lower than the biocrude oil produced from whole microalgae biomass. Furthermore, the reported price of LEA was lower than the whole microalgae biomass; hence, LEA could be a potential feedstock for producing microalgal biocrude oil.

### *4.6. Microalgal Pyrolysis Oil*

The cost of producing pyrolysis oil is reported to be higher than the HTL-derived biocrude oil (Table 8) due to the inclusion of additional unit processes such as mechanical and thermal drying of microalgae biomass. As shown in Figure 14, the cost of procuring equipment for pyrolysis and mechanical drying unit contributed majorly to Capex as a fixed capital investment. Furthermore, taxes, insurance, depreciation, and land could have added to fixed capital investment; direct installation and commissioning of pyrolysis mechanical dryers also contributed heavily to Capex.

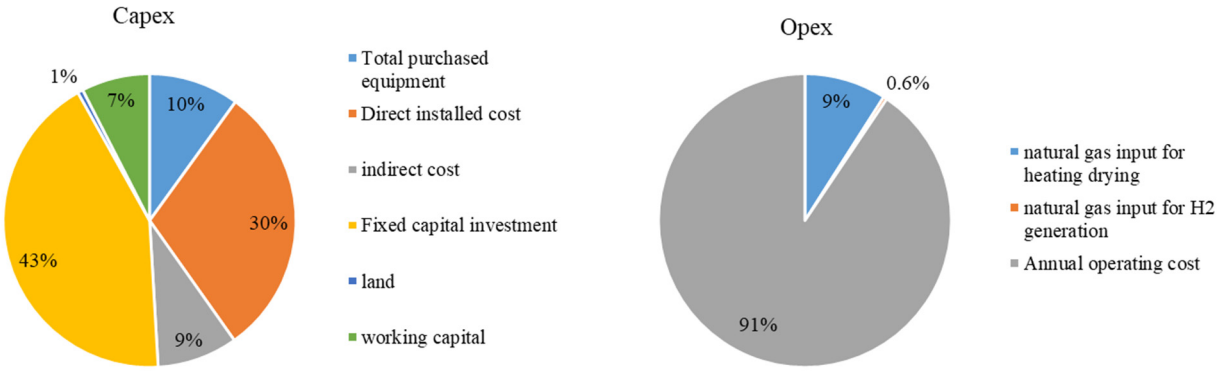



**Figure 14.** Cost distribution for producing 21.4 million gal/yr pyrolysis bio-oil from microalgae biomass (83.8% of the total cost was utilized as Capex and 16.1% as Opex) [176].

The plant management and drying of the remnant microalgae feedstock to 10% moisture content were the major contributors to Opex. Natural gas as fuel for unit operations such as drying, pyrolysis, Benzene-Toluene-Xylene (BTX) removal, fractionation, upgrading, and storage of pyrolysis oil contributed 9.6% of Opex [176]. Table 8 shows a minimum difference of 0.9 US$/L between pyrolyzed bio-oil produced from wastewater-cultivated microalgae and *Chlorella* sp. cultivated in freshwater. Alternative biomass drying options (e.g., solar drying, industrial waste heat, etc.) and upgrading of pyrolytic bio-oil may increase the chances of microalgal pyrolysis oil being used as an automotive biofuel commercially [182].

### *4.7. Microalgal Bioethanol*

An earlier study reported TEA for bioethanol production from microalgae cultivated in an open raceway pond over 3.94 ha (Table 8) [164]. As shown in the Figure 15, the Capex and Opex were based on a process for producing bioethanol using separate hydrolysis and fermentation (SHF). The total direct cost is comprised of the total equipment purchased, installed, and commissioned. Piping, electrical, and instrumentation charges are also added to the total direct cost. Indirect cost contribution towards Capex came from engineering, contingency, fees, and other expenses. The major cost of Opex was from microalgae cultivation, extraction, hydrolysis, fermentation, and distillation unit operations. Other than operational costs, the additional contributing cost to Opex was the salvage value of the bioethanol plant. Assuming the project lifetime was 20 years, the salvage value was depreciated as a cost towards Opex. The cost of bioethanol production could vary between 1.3 to 19.4 US$ $gal^{-1}$. The cost variation is due to different case study scenarios selected for the TEA studies. Further support in the form of subsidies, tax credits, and mandatory bioethanol blending policy could reduce the microalgal bioethanol price.

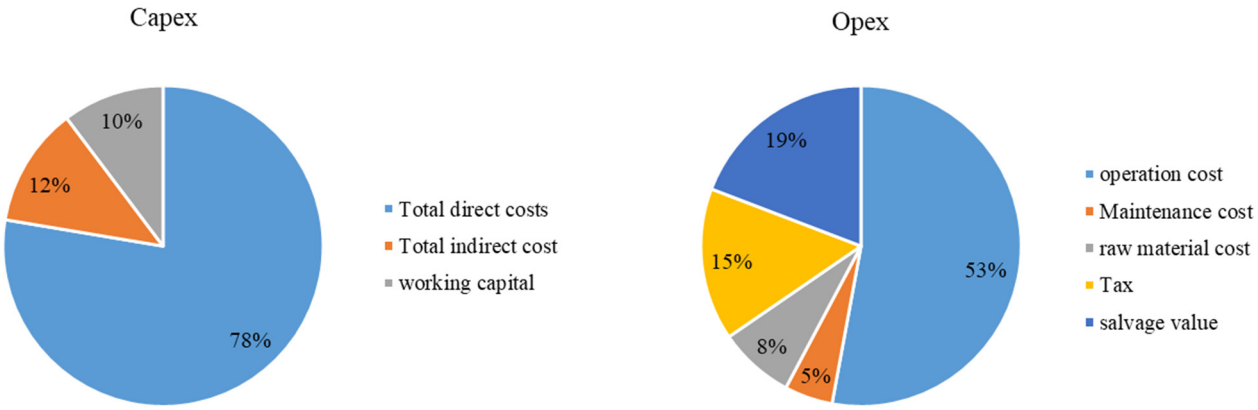



**Figure 15.** Cost contribution for producing bioethanol from microalgae biomass (29.9% of the total cost was utilized as Capex and 70.02% as Opex) [164].

As shown in Table 9, the higher heating values (HHV) of microalgal biofuels could vary based on the composition (i.e., the presence of hydrocarbons, aromatics, and oxygen and nitrogen-containing hetero-functional compounds). Unlike liquid fuels, gaseous biofuels have higher calorific values; however, storing and transporting gaseous fuels is difficult.

**Table 9.** Microalgal biofuel process technology and energy content as higher heating value.

| Microalgal Biofuel | Microalgae Strain | Processing Technology | Higher Heating Value (HHV) (MJ/kg) | References |
|---|---|---|---|---|
| Biodiesel | *Nannochloropsis salina* | Transesterification reaction | 40 | [183] |
| Bioethanol | - | Fermentation | 20.7 | [184] |
| Pyrolytic bio-oil | *Scenedesmus obliquus* | Noncatalytic pyrolysis | 36.99 | [80] |
| Biocrude oil | *Chlorella vulgaris*, *Chlorella sorokiniana*, *Scenedesmus simris* | Hydrothermal liquefaction | 35.79 | [185] |
| Biomethane | *Chlorella* sp. | Anaerobic fermentation | 39.8 MJ/m$^3$ | [186] |
| Biohydrogen | *Chlorella* sp. | Anaerobic fermentation | 12.74 MJ/m$^3$ | [186] |
| Biojet fuel | *Schizocytrium* sp. | Transesterification, separation of ethyl esters by short path distillation, deoxygenation, and hydrocracking | 46.9 | [97] |

Among the various liquid and gaseous biofuels shown in Table 9, biojet fuel has the maximum HHV value due to severe hydrotreating and refining. Comparatively, the HHV value of pyrolytic and biocrude oils could be improved further by catalytic hydrotreatment, similar to hydrotreatment used in bio-jet fuel production. Bioethanol has the lowest HHV compared to other fuels (Table 9); therefore, it could be used as an additive in gasoline fuel as an octane booster [187].

As evident from TEA described in this study, most techno-economic (TEA) studies primarily focus on the production of lipid or carbohydrate-rich biomass for biofuel production. Cultivation of lipid and carbohydrate-rich biomass adversely impacts microalgal biofuels' economic viability due to higher cultivation residence times, low biomass productivity, and higher harvesting and processing costs [188]. Therefore, TEA of protein-rich microalgae biomass cultivated in seawater and wastewater for biofuel production using technologies such as HTL, pyrolysis, and anaerobic digestion with integrated biorefinery options could offset the cost of microalgal biofuel production [188].

## 5. Recent Advances, Challenges, and Future Directions for Microalgae-Based Biofuels

Consistency in microalgal feedstock production, both in terms of productivity and quality, needs to be studied over a long period of time. Although more than 30,000 microalgal strains

have been documented, only a limited number of them have been studied extensively [189]. More efforts should be directed toward isolating new strains and developing robust screening methods for studying their desired properties. Nitrogen content in the microalgal biomass could be as high as 10% [190]. However, nitrogen content in microalgal biofuel would be very minimal, if present at all. Therefore, nitrogen-rich waste streams could be utilized together with nitrogen recycling from microalgal biomass to offset the cost and energy balance of microalgal biofuels. Furthermore, adopting a biorefinery approach would be required to make microalgal biofuels a reality in the future, irrespective of the microalgal strain selected for biofuel production. Microalgal genetic engineering technologies such as proteomics, metabolomics, CRISPR editing, riboswitching, and optoswitching techniques could offer enhanced microalgal biomass quality and yield with integrated biorefining options [191].

### *5.1. Microalgal Biodiesel*

Biodiesel synthesis using conventional homogenous catalyzed reaction leads to soaps forming, lower yields, and additional dry or wet washing for FAME purification. Furthermore, after a certain duration of repeated use, the heterogeneous catalyst gets deactivated [192]. Therefore, more recently, supercritical noncatalytic solvent systems have been developed, wherein a mixture of a solvent (e.g., ethanol, methanol, etc.) and microalgal lipids or microalgal biomass are subjected to an elevated temperature ranging from 230 to 400 °C. Biodiesel yields from *Nannochloropsis* and *Spirulina platensis* biomass using non-catalytic processes were 84.2 and 99.3%, respectively [61,193]. More recently, biodiesel has been synthesized using a non-conventional catalyst such as a nanocatalyst and ionic liquids [56,62]. Nanocatalyst has been reported to efficiently convert a varying percentage of FFA in microalgae lipid [56], while ionic liquid can perform the dual function of breaking down microalgal cells and converting triacylglycerol (TAG) and FFA to biodiesel [62]. Future perspectives for producing biodiesel from microalgae would be, (1) cultivating lipid-rich marine microalgae strains, especially cyanobacterial strains, for ease of lipid extraction, (2) screening of microalgae that can be cultivated in marine and wastewater, thereby reducing the freshwater footprint, and (3) cultivating microalgae strains that would have high-value pigments (carotenoids, xanthophylls, phycobiliproteins) or essential polyunsaturated fatty acids. The pigments could initially be separated to offset the biodiesel production cost [194]; similarly, omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in microalgae lipids could initially be converted to fatty acid ethyl esters (FAEE) and later can be separated using short path distillation or a wiped film evaporator [195]. Next, the residual saturated FAEE could be used as biodiesel. Since polyunsaturated fatty acid ethyl esters (PUFAEE) and high-value pigments can be used in food and feed applications, their separation could improve the economic feasibility of the biodiesel production process [196].

### *5.2. Microalgal Bioethanol*

Although producing bioethanol from microalgae has shown promise, it still faces economic and sustainable barriers to commercialization as a market fuel. Therefore, the technologies and findings need to be efficiently established in the future to remove the obstacles surrounding microalgal bioethanol. The bioethanol yield from microalgae has been reported to be higher, i.e., 15,000 gal/acre, compared to other terrestrial plants and lignocellulosic feedstocks [197]. Bioethanol production from marine microalgae has also been recently emphasized to reduce freshwater footprint [197]. Breaking down microalgal cells for bioethanol production has been a challenge; biological pretreatment techniques are now being applied to microalgae, wherein the bioethanol yields have shown promising results. Although the recalcitrant cell wall composition hinders effective biological treatment, further research efforts would be required to optimize the biological pretreatment of microalgal cells. Biological pretreatment has been reported to be more sustainable and eco-friendly than some other thermomechanical pretreatment techniques [198]. Furthermore, recent research has been directed more towards developing transgenic microalgae using

synthetic biology and towards recombinant DNA technology wherein single microalgae or cyanobacteria can synthesize and secrete the desired biofuel product, i.e., bioethanol [199]. Additionally, to overcome the process cost hindrance, consolidated bioprocessing is also being developed in which fungal enzymes are used in the pretreatment step for simultaneous saccharification and fermentation in a single reactor [200].

### *5.3. Microalgal Biomethane*

More recently, cascade-type microalgal production systems have been developed for achieving high microalgae growth rates, coupled with effective pretreatment methods so as to produce grid-quality biomethane [201]. Advanced molecular biology techniques, such as fluorescence in situ polymerization and restriction fragment length polymorphism to identify microbial consortia involved during the anaerobic digestion process, are currently being developed [202]. Future work should focus more on developing methanogenic bacterial strains dominating anaerobic digestion. The dominant methanogenic bacteria that utilize the acetoclastic methanogenesis pathway to increase biogas production rates need to be screened and used in AD [203,204]. Furthermore, integrating microalgae production in scaled-up wastewater systems practice needs to be undertaken on a wider scale. Microalgae preconcentrating techniques, such as membrane separation, should be developed to obtain biomass slurries with solid content ranging from 2–6%. Techniques that could effectively reduce ammonia toxicity and enhance cell biodegradability for an efficient AD process also need to be addressed. State-of-the-art engineered and designed anaerobic digesters should be developed for achieving a steady, high throughput biomethane from microalgae biomass.

### *5.4. Microalgal Biocrude Oil*

The presence of oxygenated and nitrogenous compounds in biocrude oil significantly hinders the commercialization of HTL-based microalgal biocrude oil [205]. Most importantly, for commercializing HTL biocrude from microalgae biomass, in situ HTL reaction coupled with hydrogen and deoxygenating catalyst needs to be developed. For example, zeolite catalysts and hydrogen have been found effective in the deoxygenation and denitrogenation of biocrude oil [206]. Future studies on computational fluid dynamics, kinetic modeling, and heat and mass transfer reactions that occur during HTL-coupled hydrotreating reactions need to be understood for effective experimental designs for obtaining a highly deoxygenated biocrude oil. Furthermore, recent studies are more focused on developing microalgal HTL process coupled with the separation of unwanted and value-added metabolites to improve the economic feasibility of the HTL process. For example, in a recent study, HTL of a marine cyanobacterium was conducted in which carbohydrates and pigments were removed before the HTL process [70]. Similarly, another study reported the removal of microalgal proteins to offset the cost of biocrude oil production; the projected cost of biofuel was 0.5 US$/L [207].

### *5.5. Microalgal Biohydrogen*

Pretreating microalgae for biohydrogen production has been a challenge, and therefore the following developments are currently being studied: (1) pretreatment steps with high carbohydrate extraction efficiency; (2) cost and energy efficient pretreatment steps; (3) continuous processing of wet microalgal biomass; and (4) improving the ease of scaling up on an industrial scale [208]. Recent studies have primarily focused on producing biohydrogen through a dark fermentation process using thermal and chemical techniques [209]. Furthermore, microalgal strains of industrial importance that can accumulate 70% carbohydrates are being developed by stressing during the cultivation process and through genetic engineering [210,211]. The fermenting bacteria should also be selected such that these are capable of efficiently converting polysaccharides and simple sugars to biohydrogen.

Microalgae cell lysis is essential for biohydrogen production. However, the lysis of rigid microalgae cells has been challenging for decades. Green solvents, such as supercritical fluids and ionic liquids, are currently being tested for lysing microalgal cells [212]. In

addition, algicidal compounds from certain bacterial strains are being developed to lyse microalgae cells for biohydrogen production [208].

### *5.6. Microalgal Pyrolysis Oil*

Pyrolysis oil from microalgae has been reported to possess poor fuel properties [213]. Co-pyrolysis of microalgae with other feedstocks has been recently developed, along with the use of a catalyst to reduce oxygenated and nitrogenous compounds [81]. A major bottleneck in the microalgal pyrolysis process is the use of dry microalgae as feedstocks; dewatering and drying increase the cost of pyrolysis oil. Additionally, using high temperatures and scrubbers increase the cost of microalgal pyrolysis oil. Recently, an integrated approach was suggested, wherein the protein from algae biomass could be extracted as an ingredient for animal feed and dietary supplements, whereas the polysaccharides could be separated and used for bioethanol production. The remnant biomass could then be pyrolyzed to produce bio-oil and fuel gas [214].

### *5.7. Microalgal Biojet Fuel*

Currently, producing biojet fuel from microalgae biomass is challenging and expensive. A recent study investigated biojet fuel production from a lipid-rich heterotrophic microalga, *Schizochyrtium* sp. [97]. A high-value docosahexaenoic acid (DHA) was separated from its lipid extract, and then the remaining lipid fraction was converted to biojet fuel. The price of DHA could offset the high cost of heterotrophic cultivation. Although *Schizochytrium* sp. is commercially grown as a source of DHA, bio-jet fuel production from its remaining oil would be limited due to the small market demand for DHA.

In the near future, removing value-added compounds from microalgae biomass could make bio-jet fuel production from microalgae feasible. Currently, the two most favorable routes for aviation fuel production are HTL and hydro-processed esters and fatty acids (HEFA) processes [215,216]. Future developments could include catalytic hydrotreating of microalgal pyrolytic oil to produce a high aromatic drop in fuel. Developing a catalyst that could produce selective aromatic hydrocarbons is a prerequisite for bio-jet fuel.

Lipid-rich microalgal strain is a prerequisite for bio-jet production in the HEFA process. However, the production of lipid-rich microalgal biomass is counterproductive [217], and extracting the lipid from the harvested biomass is energy-intensive [218]. Unless other value-added metabolites could be marketed in large volume, the application of the HEFA process would be limiting. On the contrary, there is great potential for the catalytic HTL process to convert microalgae biomass to bio-jet fuel. For example, whole microalgal biomass or high-value metabolites extracted residual biomass could be subject to Pd and Pt catalytic HTL to produce biocrude oil containing bio-jet fuel fractions [219].

## 6. Conclusions

Microalgae cultivation must be practiced on a large scale in wastewater or seawater on nonarable lands in open raceway ponds. Nitrogen fertilizer is one of the major inputs in microalgal biomass production. Since nitrogen is not a desired element in the microalgal fuel, technologies should be developed to recover and recycle the nitrogen from microalgae biomass. Integrating nitrogen-rich waste streams (e.g., wastewater, agro waste, etc.) in microalgal cultivation could lower the cost and environmental footprint of biofuel production.

Harvesting of microalgal biomass and its subsequent drying pose challenges to producing biofuels. It is essential to cultivate specific microalgae which are capable of producing high-value metabolites to offset the energy and cost of biomass harvesting. Because of excessive energy demand in biomass drying, focus should be given to the extraction of high-value metabolites and conversion to biofuels from wet microalgae biomass.

To improve the higher heating value and net energy ratio of biocrude oil and pyrolytic bio-oil from microalgae, continuous catalytic hydrothermal or thermochemical reactions coupled with catalytic hydrotreating need to be developed. Furthermore, the short path

distillation technique should be developed further to reduce the cost and improve the net energy ratio of biodiesel and biojet fuels from microalgal oil.

For obtaining realistic data for techno-economic studies, microalgae must be cultivated and harvested on a large scale (e.g., pre-commercial scale) with an integrated system for producing high-value products. Governmental policies and subsidies (e.g., carbon dioxide capture and utilization) should support microalgae biomass production for biofuel synthesis.

Genetic engineering, recombinant DNA technologies, producing microalgae in the heterotrophic mode, and cultivation of microalgae in wastewater and seawater must be encouraged and developed. As applying these techniques and cultivation practices could make microalgae produce valuable metabolites, extracting these metabolites could indirectly offset the microalgal biofuel production cost, thereby making microalgae biofuel sustainable in the near future.

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