energies

MDPI

Review

Progress on Conventional and Advanced Techniques of In Situ Transesterification of Microalgae Lipids for Biodiesel Production

Fazril Ideris [1,*], Mohd Faiz Muaz Ahmad Zamri [1], Abd Halim Shamsuddin [1], Saifuddin Nomanbhay [1,*], Fitranto Kusumo [2], Islam Md Rizwanul Fattah [2] and Teuku Meurah Indra Mahlia [2]

[1] Institute of Sustainable Energy, Universiti Tenaga Nasional, Jalan IKRAM-UNITEN, Kajang 43000, Selangor, Malaysia
[2] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, Faculty of Engineering and Information Technology, University of Technology Sydney, Ultimo, NSW 2007, Australia
* Correspondence: fazril@gmail.com (F.I.); saifuddin@uniten.edu.my (S.N.)

Abstract: Global warming and the depletion of fossil fuels have spurred many efforts in the quest for finding renewable, alternative sources of fuels, such as biodiesel. Due to its auxiliary functions in areas such as carbon dioxide sequestration and wastewater treatment, the potential of microalgae as a feedstock for biodiesel production has attracted a lot of attention from researchers all over the world. Major improvements have been made from the upstream to the downstream aspects related to microalgae processing. One of the main concerns is the high cost associated with the production of biodiesel from microalgae, which includes drying of the biomass and the subsequent lipid extraction. These two processes can be circumvented by applying direct or in situ transesterification of the wet microalgae biomass, hence substantially reducing the cost. In situ transesterification is considered as a significant improvement to commercially produce biodiesel from microalgae. This review covers the methods used to extract lipids from microalgae and various in situ transesterification methods, focusing on recent developments related to the process. Nevertheless, more studies need to be conducted to further enhance the discussed in situ transesterification methods before implementing them on a commercial scale.

Keywords: transesterification; microalgae lipids; biodiesel production; wet microalgae; lipid extraction; in situ transesterification

Citation: Ideris, F.; Zamri, M.F.M.A.; Shamsuddin, A.H.; Nomanbhay, S.; Kusumo, F.; Fattah, I.M.R.; Mahlia, T.M.I. Progress on Conventional and Advanced Techniques of In Situ Transesterification of Microalgae Lipids for Biodiesel Production. *Energies* **2022**, *15*, 7190. https://doi.org/10.3390/en15197190

Academic Editor: Attilio Converti

Received: 2 September 2022
Accepted: 26 September 2022
Published: 29 September 2022

1. Introduction

Since the beginning of the industrial revolution, fossil fuels have been used for meeting a majority of the world's energy demands. Petroleum (both heavy and light crude oils), coal, and natural gas contribute to approximately 81% of the world's energy demands in 2017, a percentage that held steady for the past three decades [1,2]. Apart from that, the world is experiencing an upward trend in global warming. It is projected that the average surface temperature of the Earth will continue to increase by about 1.2 °C (0.9–1.6 °C) in the year 2200, with respect to the year 2000 [3]. One of the main contributors to global warming is CO_2 that originated from the emission of anthropogenic greenhouse gas (GHG) when fossil fuels are burned [4].

It has been noted that both issues of fossil fuel depletion and global warming are closely related. Hence, approaches that can solve these issues simultaneously should be implemented. One of the ways to address this is via renewable energy in the form of biofuel [5]. Interest in biofuel has grown from only being dominated by small operators and a few countries to a globally traded commodity amongst industry players, including major oil producers [6]. Two types of biofuels, namely biodiesel and bioethanol, have come to the forefront as the most viable options to replace diesel and gasoline, respectively—since

they are considered as a 'direct-pour' alternative fuel that can be used in today's existing automobiles without needing much or any modifications [7,8]. Diesel engine manufacturers such as Volvo and Mercedes have approved the usage of biodiesel blend in their engines [9]. Due to the soaring price of fossil fuels, there are even predictions that future diesel engines will be entirely operated using pure biodiesel [10]. The term biodiesel usually refers to the ester products produced from the transesterification of lipids (triglycerides) contained in the biomass with alcohol such as methanol or ethanol [11,12]. Normally, methanol is a preferred choice of alcohol to be used in the transesterification reaction, since it is about 24% cheaper than ethanol [13]. Conventionally, syngas is used as the raw material to produce methanol, using copper as catalyst [14]. As much as 11.2 kWh of energy (mainly obtained from coal and natural gas) is required to produce 1 kg of methanol [15,16]. However, methanol can also be produced via CO_2 hydrogenation [17]. This greener pathway can be achieved by using electricity produced from wind power, electrolysis of water, and captured CO_2, where GHG emissions are reduced by 59% as compared to the conventional method [18,19].

Due to biodiesel's lower calorific value as compared to diesel, the engine torque and power for biodiesel are lower by approximately 8% and 7%, respectively [20,21]. However, for B20 fuel blend (20% biodiesel), this reflects about a 1–2% inferiority in engine power, and most users admit that there is no noticeable difference in performance [22]. Currently, the cost to produce biodiesel is substantially higher than the cost to process fossil fuels. Therefore, there is a pressing need to reduce the cost associated with the entire biodiesel production process for it to at least compete with fossil fuels [23–25]. Among the ways to reduce the costs are the careful selection of high potential but cheap feedstock, usage of better engineering devices, simpler and better processing techniques, and optimization of the overall process [26,27]. A title search for reviews with the keywords "in situ transesterification" and "microalgae" resulted in only two previous review articles [28,29]. The previous study by Kim et al. [29] focused on reviewing the in situ transesterification to biodiesel using wet microalgae. In addition, they also reviewed the techno-economic analysis of biodiesel production of wet *Nannochloropsis gaditana*. Another study by Salam et al. [28] discusses the pathways to the effective utilisation of methanol and residual algae produced during in situ transesterification. They also reviewed the factors that affect the process along with the ways to improve its economy through a new integrated process. Park et al. [30] termed in situ transesterification as direct transesterification and discussed the then advances in biodiesel conversion from microalgae both by the conventional two-step process as well as by direct transesterification.

This article reviews the state-of-the-art in situ transesterification of microalgae to biodiesel. This paper covers the current methods for extracting lipids from abundantly available microalgae feedstock, focusing mainly on examining various recent processing techniques involving in situ transesterification for the biodiesel production from microalgae. Factors such as cost, tediousness, and current challenges are carefully evaluated for each method. The usage of homogeneous and heterogeneous catalysts, carbon-based catalysts, biocatalysts, and advanced methods utilising state -of-the-art co-solvents, microwave irradiation, ultrasonic, and supercritical fluids conditions were extensively discussed with an emphasis on each method's commercial viability for the production of biodiesel. The advantages and disadvantages of each method are also discussed thoroughly, with the intention of evaluating its suitability for scaling-up purposes. This article also discusses some of the hindrances as well as the future directions for in situ transesterification. Recommendations of potentially viable in situ transesterification methods for scaling up purposes are also made accordingly.

2. Microalgae as a Feedstock for Biodiesel Production

Various feedstocks, such as plants [31,32], animal fats [33], waste materials [34,35], and microalgae [36], have been utilised in biodiesel production. Climate, topography, soil conditions, and economic activities are among the factors that influence the selection of

ready feedstock in a specific region [37,38]. For example, palm oil and coconut oil are primarily used in tropical countries, rapeseed and sunflower oil in Europe, while soybean, peanut, and animal fats are used in North America [39,40]. In addition, it is a good idea to have a wide selection of feedstocks so that biodiesel production does not depend only on a handful of options. This is the main reason why the extensive effort in improving the process and finding new potential feedstocks needs to be conducted continuously. The lipid content in the feedstock is one of the many factors considered in its usage for biodiesel production [41,42]. It has been reported that 1 Ha of an algae farm on wasteland can produce over 10- to 100-times of oil and bioethanol yield as compared to any other known source of oil crops [43]. The lipid contents for various feedstock that are commonly used to produce biodiesels are shown in Table 1.

Table 1. Oil content, oil yield, land area requirement, and biodiesel productivity for several feedstocks used in biodiesel production [44–46].

Feedstock	Oil Content (% of Dry Weight)	Oil Yield (L Oil/ha/Year)	Land Area Requirement (m^2/Year/kg Biodiesel)	Biodiesel Productivity (kg Biodiesel/ha/Year)	References
Castor (*Ricinus communis*)	48	1307	9	1156	[44]
Corn/maize (*Zea mays* L.)	44	172	66	152	[44]
Hemp (*Cannabis sativa* L.)	33	363	31	321	[44]
Soybean (*Glycine max* L.)	18	636	18	562	[44]
Jatropha (*Jatropha curcas* L.)	28	741	15	656	[44]
Camelina (*Camelina sativa* L.)	42	915	12	809	[44]
Canola/rapeseed (*Brassica napus* L.)	41	974	12	862	[44]
Sunflower (*Helianthus annuus* L.)	40	1070	11	946	[44]
Peanut oil (*Arachis hypogaea* L.)	50	1059	-	1425–1782	[45,46]
Hazelnut (*Corylus avellana)*	51–75	-	-	1000	[46]
Palm oil (*Elaeis guineensis*)	36	5366	2	4747	[44]
Walnut (*Juglans regia)*	51–72	-	-	780–1750	[46]
Microalgae (low oil content)	30	58,700	0.2	51,927	[44]
Microalgae (medium oil content)	50	97,800	0.1	86,515	[44]
Microalgae (high oil content)	70	136,900	0.1	121,104	[44]

From Table 1, it is clearly seen that microalgae show an immense potential to be utilised as a feedstock in producing biodiesel. Microalgae have attracted much attention—as early as the 80s—and are hailed as one of the most promising feedstocks to displace fossil fuels entirely [47]. With its capacity to produce more oil per unit area when compared to other types of feedstocks, coupled with its unique characteristic to not be entangled with the food versus fuel dilemma, microalgae are regarded as a robust plant that can be cultivated in the most hostile environments on Earth [48]. In addition, microalgae cultivation plants can be located near places such as power plants and animal farms in the effort to mitigate CO_2 and treat wastewater, while at the same time turning waste to useful bioenergy [49–51]. Figure 1 shows the side elevation of a microalgae cultivation pond—known as High-Rate Algal Pond (HRAP)—utilising wastewater from domestic, industrial, or agricultural sources [52]. With its proven effectiveness, HRAP may convert wastewater treatment plants into energy producers [53].

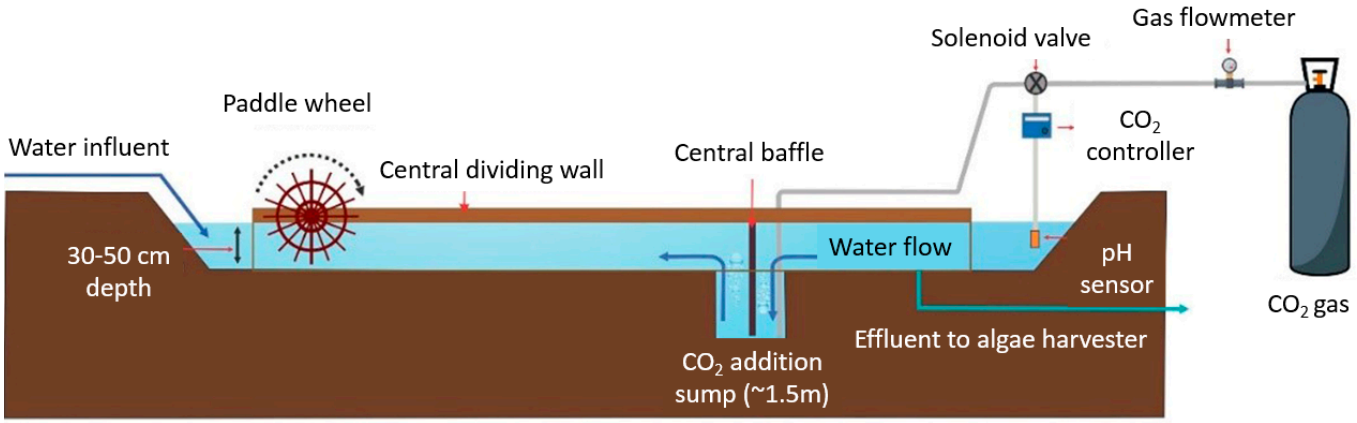

Figure 1. Side elevation of HRAP for wastewater treatment using microalgae with CO_2 addition to enhance algal growth [52].

The symbiotic process involved in treating wastewater using microalgae is illustrated in Figure 2 [54]. During the treatment process, microalgae would absorb carbon dioxide via the photosynthesis process, which would result in the production of oxygen. Nutrients such as nitrogen and phosphorus would also be absorbed by the microalgae. The produced oxygen would then be utilised by bacteria in degrading organic matter contained inside the wastewater.

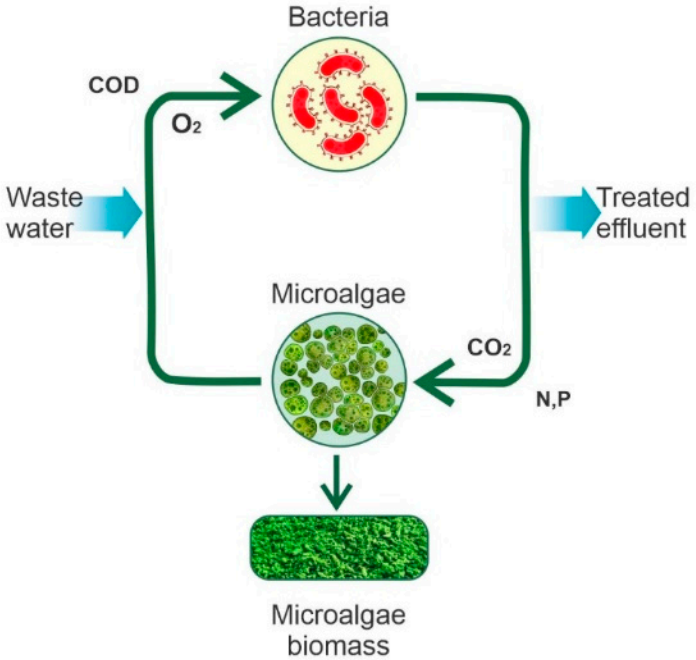

Figure 2. Symbiotic process between microalgae and bacteria in wastewater treatment [54].

The lipid contained in microalgae is known to be anywhere from 20–50% of the total dry weight [55]. It was discovered that nitrogen plays an active role in suppressing lipid synthesis in microalgae. Under nitrogen starvation, the lipid content in microalgae can reach more than 70% of its total dried weight [56,57]. From the vast number of microalgae species that have been studied, there are a few species that are recommended in the production of biodiesel due to the high percentage of lipid content. In a study involving 12 microalgae species, the best selections of microalgae based on their fatty acid profiles for biodiesel productions are *Chlorella vulgaris*, *Nannochloropsis oculata*, *Extubocellulus* sp., and *Biddulphia* sp. [58]. In terms of the ability to withstand the cultivation environment with high ambient

temperature, *Desmodesmus* sp. is recommended due to its capability to survive for 24 h at 45 °C or 8 h at 50 °C [59]. In a different study conducted on *Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis* sp., *Neochloris oleoabundans*, *Scenedesmus obliquus*, and *Dunaliella tertiolecta*, it was found that two particular species, namely *Neochloris oleoabundans* and *Nannochloropsis* sp., were concluded to be the best options in terms of quality and quantity of biodiesel produced—where both species yielded more than 50% in lipid content under nitrogen starvation [60]. As expected, microalgae species that possess positive traits, such as being easy to cultivate and having a high rate of sedimentation and high lipid content, are usually preferred for biodiesel production [59,61]. Table 2 illustrates the lipid content, lipid productivity, volumetric productivity, and areal productivity for several microalgae species (adapted from [50]).

Table 2. Lipid content, lipid productivity, volumetric productivity, and areal productivity for microalgae species [50].

Microalgae Species	Lipid Content (% Dry Weight Biomass)	Lipid Productivity (mg/L/Day)	Volumetric Productivity of Biomass (g/L/Day)	Areal Productivity of Biomass (g/m^3/Day)
Ankistrodesmus sp.	24.0–31.0	-	-	11.5–17.4
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.007	–
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorococcum sp.	19.3	53.7	0.28	–
Crypthecodinium cohnii	20.0–51.1	–	10	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Ellipsoidion sp.	27.4	47.3	0.17	–
Euglena gracilis	14.0–20.0	–	7.70	–
Haematococcus pluvialis	25.0	–	0.05–0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	–	0.32–1.60	–
Monodus subterraneus	16.0	30.4	0.19	–
Monallanthus salina	20.0–22.0	–	0.08	12
Nannochloris sp	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0			8.8–21.6
Oocystis pusilla	10.5	–	–	40.6–45.8
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	–
Skeletonema costatum	13.5–51.3	17.4	0.08	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	–

The process of producing biodiesel from microalgae is summarised in Figure 3. Despite its huge potential, the utilisation of microalgae in the production of biodiesel comes with some challenges. One of the issues is the high cost associated with biodiesel production from microalgae, which are $2.59 and $2.00 per litre through raceway and photobioreactor cultivations, respectively [62]. One of the main aspects that contributes to the high cost is the extraction of the intercellular encapsulated lipid, wherein the tough, thick wall of the cells needs to be disrupted properly prior to the extraction. However, cell disruption cannot be done via conventional physical methods but has to be performed through mechanical, biological, or chemical methods by using solvent [63]. It is reported that the cost of the lipid extraction process can be as high as 90% of the total biodiesel production cost [64]. For traditional methods that involve lipid extraction from dried microalgae, the harvested microalgae are to be dried via solar drying, thermal dryer, hot flue gas, or by using an oven. A thermal dryer powered by natural gas or electricity is the most common practice at the commercial scale [65]. However, the dewatering process alone would require a substantial amount of energy in addition to being very time-consuming. One thousand gallons of dewatering is needed in order to obtain a gallon of dry algal biomass, not including the pre-treatment process involved before the extraction of lipid [66], and the cost involved in the dewatering process is reported to account for up to 30% of the total production [67]. Apart from that, the process of extracting lipid from dried microalgae usually involves toxic or carcinogenic solvents such as chloroform and dichloromethane, making it unfeasible to be commercialized on a large scale. The typical experimental procedures for lab-scale production of biodiesel from microalgae is shown in Figure 4, where the organic solvent mixture is used during the lipid extraction process [68].

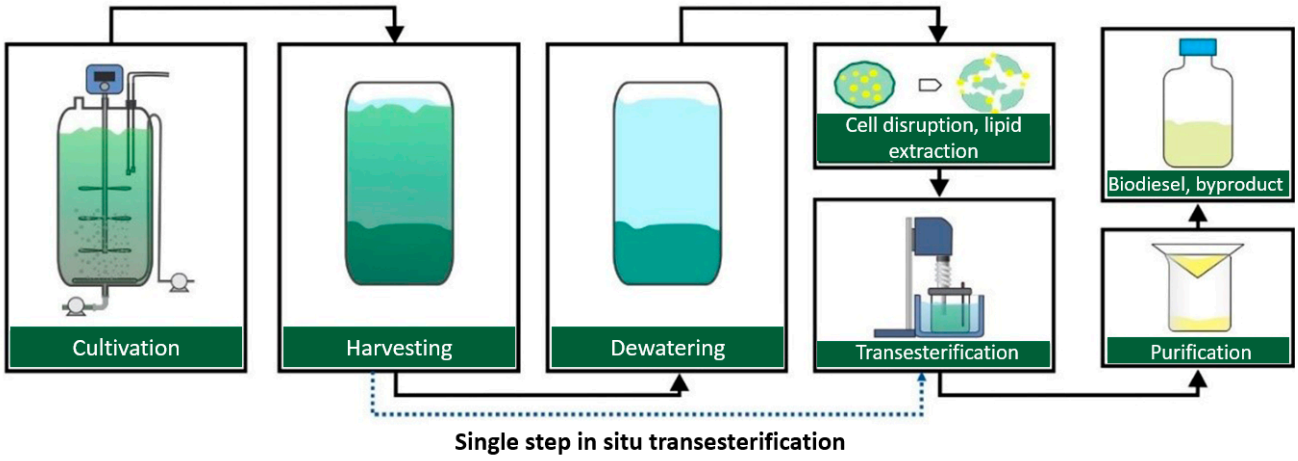

Figure 3. Processes involved in the production of biodiesel from microalgae. In situ transesterification is done generally by mixing alcohol and catalyst with the wet microalgae, allowing the production of biodiesel without undergoing dewatering and lipid extraction processes individually (adapted from [69]).

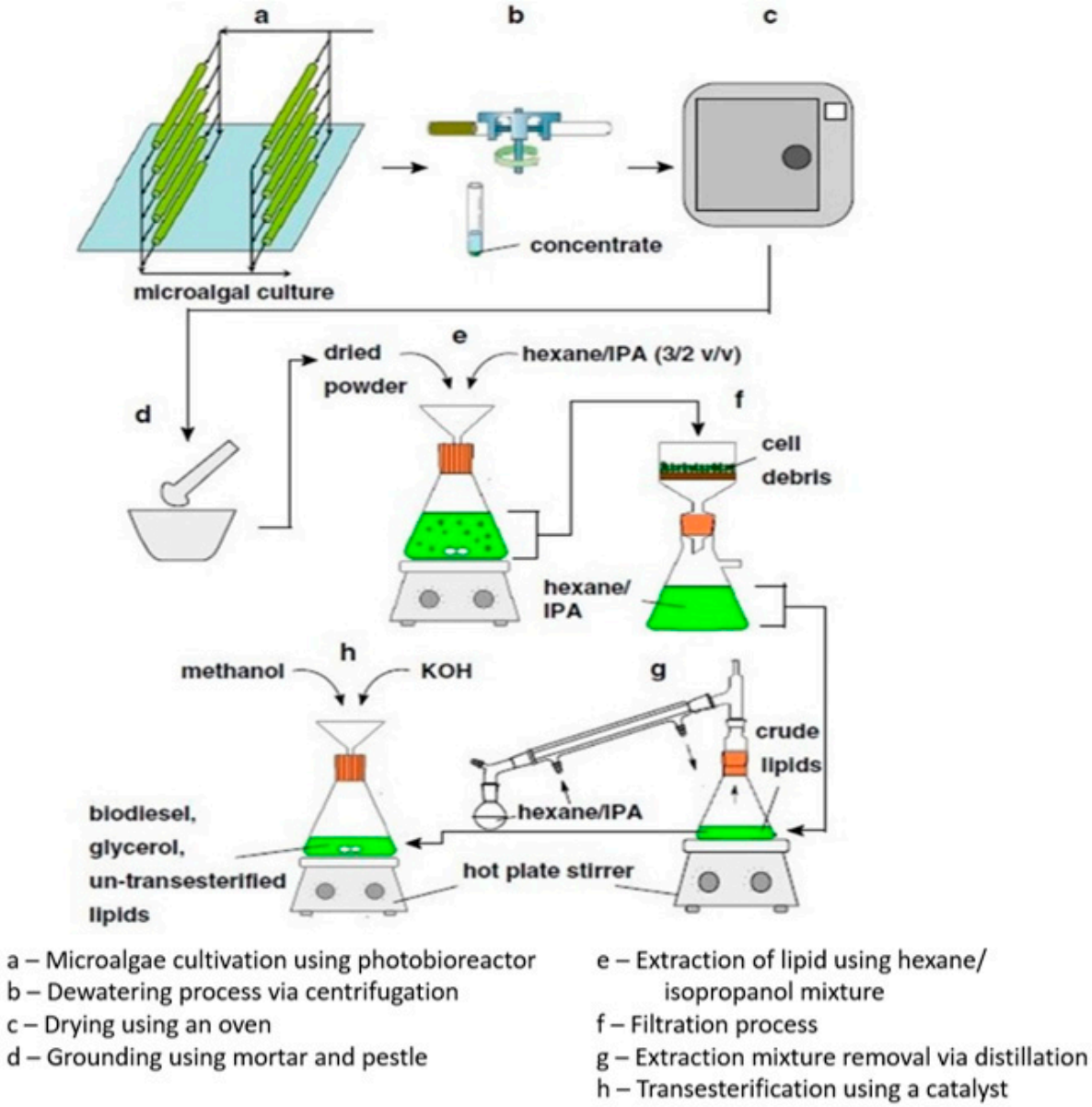

Figure 4. The typical laboratory-scale experimental procedures for producing biodiesel from microalgae biomass using the organic solvent mixture for lipid extraction [68].

The tediousness and high production costs are among the main hindrances in the development of using microalgae as a reliable feedstock to produce biodiesel [63,70]. Hence, most of the time, the production of biodiesel from microalgae has been limited to a laboratory scale due to all these challenges [28]. There are even doubts about whether microalgae would ever be an economically suitable feedstock for biodiesel production [71,72]. The only way for it to be economically viable is via improved engineering involving both upstream and downstream sectors [73]. One of the ways to improve the process is by extracting lipid from wet microalgae, hence circumventing the drying process [74]. The extracted lipid is then converted to biodiesel via a conventional transesterification process. Another option is via direct or in situ transesterification, as shown in Figure 3, wherein the extraction of lipids from wet biomass and the transesterification process are done in a single step [70].

3. Microalgae Cell Disruption

The small size of the microalgae cell and the complexity of the wall's structure itself is very different compared to other terrestrial plants [75]. The cells are also generally

quite resistant to chemical or mechanical stresses due to the eukaryotic nature of microalgae [76]. In addition, the variation of the cell wall is so vast since it depends on multiple factors, including the cultivation environment, species (approximately 72,500 species via conservative estimation), and nutrient supply, up to a point where the cell would expand/contract—and consequently, assumes various shapes depending on the overall environment [77–79]. The variation and composition of the intercellular wall for six microalgae, namely *Neochloris oleoabundans*, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Haematococcus pluvialis*, and *Nannochloropsis gaditana*, are illustrated in Table 3.

Table 3. Cell wall characteristic for several microalgae species.

Species	Cell Wall Characteristics	References
Neochloris oleoabundans	- two distinct layers - made from 24.3% carbohydrates, 31.5% proteins - carbohydrates component consist of non-cellulosic polysaccharides	[78]
Chlorella vulgaris	- two distinct layers - the outer layer is an electron-dense wall, while the inner layer is low in density	[80]
Chlamydomonas reinhardtii	- five distinct layers - made from hydroxyproline-rich glycoproteins - made up totally from glycoproteins, with no cellulose	[81]
Dunaliella salina	- lack of rigid cell wall - the cell is isolated by a thin elastic plasma membrane	[82]
Haematococcus pluvialis	- three-layer cell wall - first layer: extracellular matrix, algaenan layer. - secondary layer: thick amorphous layer made of mannose and cellulose - tertiary layer: heterogeneous layer made of mannose and cellulose	[76,83]
Nannochloropsis gaditana	- two layers, with a cellulosic inner wall and an outer hydrophobic algaenan layer, which is formed by highly saturated aliphatic compounds	[84]

In order to release the intercellular encapsulated lipid, the thick, robust wall (which is usually made from sturdy polymers such as cellulose, hemicellulose, and pectin) of microalgae needs to be disrupted properly [85]. Due to the wide variations in the wall structure, as shown in Table 3, it is difficult to have a standard procedure for disrupting the cell wall. One method may work well for a species, but the same method might not be efficient for another. From studies, it was found that cells with more complex structures are more challenging to be disrupted, and species with a thinner wall are easier to be disrupted [86]. This process has proven to be challenging without a large amount of energy or solvent involved. Due to the lack of efficient methods, continuous studies are needed to find alternative ways of extracting the encapsulated lipid [87]. The methods for disrupting microalgae cell walls can be divided into two general paths: mechanical and non-mechanical methods, as presented in Figure 5 [76,88].

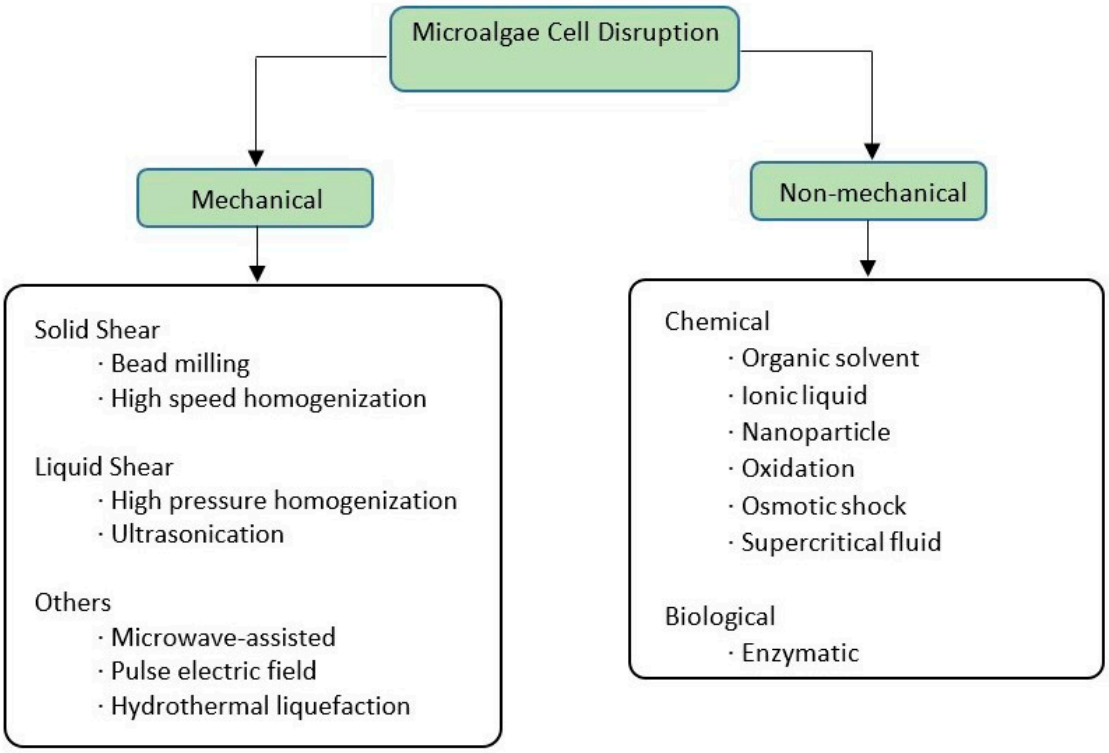

Figure 5. Various ways for disrupting microalgae cell wall [76,88].

For each of the methods presented in Figure 5 above, there is a need to scrutinize the possibility of the methods to be scaled up for commercial purposes; not only that, but the overall potential impact of each method on the environment also needs to be considered. These are among the most crucial factors in assessing the viability of its utilisation to produce biofuels commercially [89]. A summary of each method is presented in Table 4.

Table 4. Summary of various lipid extraction methods.

Methods	Advantages	Limitations	References
Bead milling	High-rate cell disruption; practical method of large scale mechanical cell disruption	Degree of disruption depends on characteristics of the bead; requires a large amount of energy in large-scale applications	[89,90]
High-speed homogenisation	High rate cell disruption, very effective; short extraction time	High energy consumption, not suitable for large scale application	[88,91]
High-pressure homogenisation	Effective, rapid disruption of cell; suitable for scaling up	Rapid disruption, but generally lower lipid yield compared to other methods; high level of cell debris is released, which complicates the separation process.	[86,92]
Ultrasonication	Short extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improved release of intracellular contents	High power consumption; difficult to scale up	[86,89]
Microwave-assisted	Relatively simple, safe, rapid, economical in lab-scale	Maintenance on a large scale is a limiting factor; prone to free radicals formation	[93,94]

Table 4. *Cont.*

Methods	Advantages	Limitations	References
Pulse electric field	No addition of chemical; low energy consumption; rapid disruption	Prone to decreasing uniformity in the electric field due to the presence of air bubbles in chamber; the solution must be free of ions	[92,95]
Hydrothermal liquefaction	High-quality biocrude is obtained, environmentally friendly due to the usage of water in the extraction	High energy requirement due to very high temperature involved, more in-depth studies are needed	[96,97]
Organic solvent	Relatively cheap, very effective, high oil yield	Residues of solvent in extracts; most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy-intensive, a large volume of solvent is required	[98,99]
Ionic liquid	Short extraction time, reusability, and high oil yield	Some ionic liquids are toxic to the environment, highly expensive	[100,101]
Nanoparticle	High efficiency, low energy requirement, reusability	Some are very expensive; synthesis cost needs to be evaluated for commercial purposes	[102]
Oxidation	An effective method; high yield, high saturated hydrocarbon products	More works need to be done for big-scale implementation	[103]
Osmotic shock	Simple extraction, low energy consumption	Generation of waste salt water, time-consuming	[50,104]
Supercritical fluid	High oil yield; non-toxic (no organic solvent residue in extracts); non-flammable	High energy consumption; expensive and difficult to scale-up	[42,105]
Enzymatic	Mild operating conditions; low energy requirement	Long process time; low production capacity	[88,106]

As seen in Table 4, most of the existing methods are not suitable or costly for scaling up, hence making them undesirable for industrial purposes. It can also be inferred that many improvements in microalgae processing are needed for large-scale biodiesel production. This is one of the factors that makes in situ transesterification an appealing option since two of the costliest processes, dewatering and microalgae cell disruption for lipid extraction, can be entirely circumvented.

4. In Situ Transesterification of Microalgae

Since microalgae are set to be one of the most promising feedstocks for biodiesel production, many efforts have been made to break the bottleneck associated with the conversion process. One of the ways to do this is by adapting a downstream process known as direct or in situ transesterification, where biodiesel is obtained directly from wet harvested microalgae without undergoing the drying and lipid extraction processes separately. During in situ transesterification, wet microalgae is mixed with alcohol in the presence of a catalyst, and occasionally co-solvents [107]. This has proven to be a favourable practice due to its simplicity from combining lipid extraction and transesterification, hence reducing the processes in producing biodiesel [108]. By circumventing the drying process, these methods would help to simplify and significantly reduce the total overall production cost, hence making microalgae a more viable feedstock in the production of biodiesel [109].

In situ transesterification for the production of biodiesel has also been applied to other feedstocks, such as palm oil [110], soybean [111], Jatropha curcas [112], castor seed [113],

rapeseed [114], linseed [115], and yeast [116]. A significant increment of research works related to in situ transesterification, especially on microbial agents (including microalgae), can be seen starting from 2011 onwards [117]. This is mainly due to the eagerness of the world to find alternatives to fossil fuels and the change in policies on renewable energies from various governments all over the world. For example, in Asia, Japan, as a developed country, is actively trying to increase the utilisation of biodiesel for the freight vehicles used by the transportation and automobile sectors in realizing its pledge of reducing its GHG emissions by 80% by 2050; while Malaysia, as a developing country, has also directly encouraged the growth of biodiesel usage by pledging to reduce the GHG emission intensity of the GDP by 45% by 2030 relative to its emissions in 2005 [118,119]. Funding in renewable energy implementations parallel to these pledges by governments has contributed a lot in the increase of interest on renewable energies [120].

In situ transesterification has proven to be an effective and economical way for both single-species [121] and mixed-species cultures of microalgae [122]. A reasonable biodiesel yield is obtained from in situ transesterification when compared to the conventional two-step extraction transesterification methods. If the ideal in situ transesterification method is employed for a specific species, it will yield a higher biodiesel yield compared to the conventional two-step esterification–transesterification method. In a comparative study involving the wet microalgae, *Schizochytrium limacinum*, in situ transesterification using solvent resulted in a 66.97% biodiesel yield compared to the 59.73% yield via conventional two-step esterification–transesterification method [123]. This is partly due to the elimination of the oil extraction step, which may result in oil loss, hence increasing the amount of biodiesel yield. Generally, in situ transesterification techniques can be categorized into conventional techniques and advanced techniques, as seen in Figure 6 (adapted from [30]).

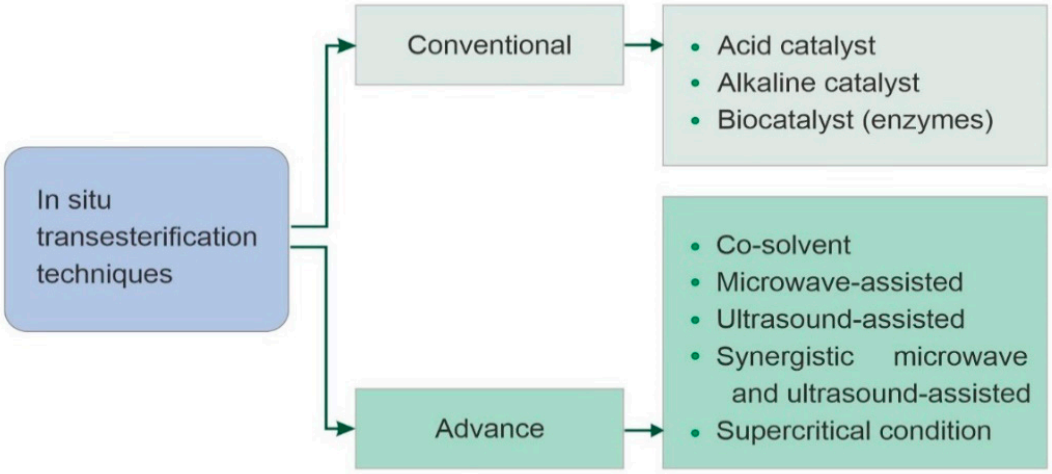

Figure 6. General classifications of in situ transesterification techniques (adapted from [30]).

4.1. Conventional Techniques for In Situ Transesterification

Conventional in situ transesterification methods, such as acid-catalyzed methods, have been explored as early as the 1980s by researchers using sunflower seeds as feedstock [124]. It was found that the acid-catalyzed method is especially effective for in situ transesterification involving feedstock with a high level of free fatty acid (FFA) and moisture content, usually in the range of more than 1% and 0.5%, respectively [30,49,117]. When this method is applied to microalgae, it has proven to be very effective since microalgae generally contain a high level of FFA [125]. In a sulfuric-acid-catalyzed in situ transesterification study involving microalgae biomass from the species of *Botryococcus braunii* with a moisture content of 7.8 wt %, a biodiesel yield of 82% was obtained when pure methanol was used in the process [126]. In another work done by Kim et al. [127], highly-wet *Nannochloropsis gaditana* microalgae were mixed with methanol and hydrochloric acid during an in situ transesterification, resulting in more than a 90% biodiesel yield at

the reaction temperature of 95 °C. In addition, it was found that the usage of chloroform as a solvent would give a better yield compared to hexane—yield of 90% and 70% for chloroform and hexane, respectively. In the same work, it was also discovered that the usage of hydrochloric acid would result in a 15 wt % higher FAME yield compared to when sulfuric acid was used. There was an interesting study to evaluate the effectiveness of ethanol and methanol during the sulfuric-acid-catalyzed in situ transesterification of *Chlorella vulgaris* by Lemões et al. [128]; it was found that a similar yield of fatty acid ethyl ester (FAEE) and fatty acid methyl ester (FAME) of approximately 11% were obtained from ethanol and methanol, respectively. Hence, the usage of ethanol, which is more sustainable and less toxic compared to methanol, could be a better choice in the greener production of biodiesel. However, there are several setbacks when homogenous acid is used as a catalyst. Longer reaction times and higher temperatures are usually needed for acid-catalyzed in situ transesterification [129]. In a study to evaluate the effect of temperature on FAME yield, it was found that the yield varied from 49.1–90.6% when the reaction temperatures were changed from 65 to 95 °C [130]. In addition, the usage of homogenous acid in the transesterification process would lead to the need for a large volume of water for removing the catalyst during the separation and purification processes [131]. There are also studies on in situ transesterification by using alkali as catalysts, wherein potassium hydroxide (KOH) and sodium hydroxide (NaOH) are the most commonly used [132,133]. However, an undesirable saponification reaction tends to occur when alkali is used as a catalyst due to the high FFA content in microalgae. This is quite contrary to other types of feedstocks, where, in most cases, alkali is a preferable choice as a catalyst since it yields a significantly higher biodiesel amount at a shorter reaction time and is generally lower in cost compared to acid [134]. Nevertheless, a decent biodiesel yield is still possible by using a significantly higher amount of alcohol during the in situ transesterification process for microalgae. In a study, a 77.6 ± 2.3 wt % biodiesel yield was obtained via in situ transesterifications of *Chlorella vulgaris* at a reaction time of 75 min by utilising sodium hydroxide (NaOH) as a catalyst, and using a methanol-to-lipid molar ratio of 600:1 while keeping the molar ratio of 0.5:1 for a catalyst to lipid [135]. In another work, a biodiesel yield of 95.5% was obtained in 10 min with the temperature kept at 60 °C by using highly-concentrated NaOH (96% *w*/*w* oil) with an ethanol-to-lipid molar ratio of 925:1 [133]. However, the high quantity of alcohol used during the in situ transesterification involving a homogenous alkali catalyst could result in high expenses that occur during the alcohol recycling process [136].

Among the main issues related to the usage of homogenous acid and alkali as catalysts during the in situ transesterification process are the separation and purification of the final products, regardless of the generally high biodiesel yield. It is difficult for the homogenous catalysts to be separated at the end of the process, and this separation phase has a notorious reputation due to its complexity and energy consumption, hence leading to significant catalyst loss and higher biodiesel costs [137]. Due to this, heterogeneous catalysts have received a lot of attention for being utilised in the in situ transesterification process. In addition to the easier separation of final products, heterogeneous catalysts are also recyclable, non-corrosive, and ecologically friendly, hence making them more favorable compared to homogenous catalysts [138]. Among the examples of heterogeneous catalysts are the group II oxides; composites of $CaTiO_3$, $CaCeO_3$, $CaZrO_3$, WO_3/ZrO_2, $H_4SiW_{12}O_{40}$, $H_3PMo_{12}O_{40}$, and $H_3PW_{12}O_{40;}$ ion exchange resins such as Amberlite-15, CT-275, and CT-269; and a carbon-based heterogeneous catalyst such as graphene oxide, etc. [139–141]. Heterogeneous acid catalysts of both Brønsted-type and Lewis-type have the ability of both acid and alkali catalysts during the transesterification process [142]. In other words, these types of heterogeneous catalysts offer a faster reaction time while avoiding the soap formation during the process. Several recent in situ transesterification works have been carried out by using these types of catalysts, and the results have been shown to be satisfactory—producing high yields compared to the usage of homogenous acid catalysts while having all the advantages discussed above [143,144]. In recent works, a heterogeneous acid catalyst CT-269 ion-exchange resin was used during an in situ trans-

esterification of *Nannochloropsis gaditana* under optimized conditions [139]. In the study, different parameters, such as the reaction temperature, the ratio of catalyst to microalgae oil, and the ratio of methanol to oil, were evaluated, and it was determined that the ratio of catalyst to microalgae oil is the most prominent factor that should be considered for producing high biodiesel yield.

Environmentally benign carbon-based catalysts such as graphene and activated carbon have also been studied extensively for the production of biodiesel [145,146]. Graphene is a plane of carbon atoms arranged in two dimensions. When graphene is wrapped in spherical or cylindrical forms, the allotropes are known as buckyball and carbon nanotubes, respectively [147]. The effectiveness of these catalysts has been attributed to their excellent properties, such as high surface area (800–1500 m^2/g for activated carbon) and high thermal stability, which usually resulted in biodiesel yields of more than 95% [141,148]. However, in most cases, a very high ratio of alcohol to oil is needed to carry out the process [149]. In a comparative study to evaluate the effectiveness of different types of carbon-based catalysts, Jun Cheng [150] compared the FAME yields obtained from several in situ transesterifications of wet microalgae biomass using graphene oxide, sulfonated graphene, sulfonated active carbon, and sulfonated graphene oxide. Even though the yield of in situ transesterification using homogenous sulfuric acid is higher than any of the yields using the carbon-based catalysts, sulfonated graphene oxide emerged as the best option in terms of biodiesel yield among all four carbon-based catalysts, with a yield of 84.6% being catalyzed by acid sulfuric.

In addition to homogeneous and heterogeneous catalysts, biocatalysts can also be used for in situ transesterification, since they operate at much lower temperatures (hence, lower energy consumption) compared to acid/alkali-catalyzed transesterification, and the separation of the final products is a lot simpler [151,152]. Available in either liquid or immobilised forms, these heterogeneous biocatalysts are also non-corrosive and considered to be less harmful to the environment [153]. Decent FAME yields have been reported for various in situ transesterification works involving biocatalysts [154,155]. In a study, 82% of the FAME yield was obtained from microalgae species of *Oedogonium* sp. via in situ transesterification with *Bacillus* sp. lipase as the catalyst [156]. However, the amount of energy taken for this process is quite significant, since the temperature was kept at 55 °C during the reaction time of 36 h. Therefore, the process can be further improved by having a lower reaction temperature or a shorter reaction time. Recently, a two-step enzymatic process on wet *Chlorella* biomass was employed by first using cellulase at a reaction temperature of 40 °C for 24 h and then by using liquid lipase TL from *Thermomyces lanuginosus* kept at 25 °C for 48 h, resulting in an 83.79% biodiesel yield [157]. This two-step enzymatic process has been proven to further reduce the energy associated with the in situ transesterification process using biocatalysts. Table 5 contains some of the recent works involving biocatalysts.

Table 5. Biodiesel yields for several biocatalysts with respect to the microalgae species.

Lipase Origin	Microalgae Biomass	Reaction Time (h)	Biodiesel Yield (%)	References
Whole cell from *Pseudomonas aeruginosa*	*Spirulina platensis*	48	87.6	[158]
Free enzyme from *Candida rugosa*	*Scenedesmus quadricauda*	24	85.7	[159]
Novozyme 435 from *Candida antarctica*	*Botryococcus* sp.	4	88	[160]
Free enzyme from *Rhizomucor miehei*	*Chlorella vulgaris*	25	90	[161]
Immobilised whole cell from *Aspergilous niger*	*Scenedesmus obliquus*	36	53.76	[153]
Novozyme 435 from *Candida antarctica*	*Aurantiochytrium* sp.	12	89.5	[162]
Immobilised lipase from *Candida antarctica*	*Chlorella vulgaris*	24	97	[163]

Another advantage of using lipase is its reusability for repeated transesterification processes. The same lipase batch can be used several times before it loses its ability as a catalyst. This is particularly important in the biodiesel industry in order to make it econom-

ically feasible. In a recent study, Raoufi and Gargari [158] reused optimized lipase obtained from Pseudomonas aeruginosa for 10 cycles in their in situ transesterification involving wet *Spirulina platensis* biomass, before the biodiesel yield fell by roughly 9% compared to the first batch. Meanwhile, CalB lipase from *Candida antarctica*—immobilised in mesoporous materials—was utilised for 7 repeated cycles in the ethanolysis of *Nannochloropsis gaditana* before losing its potency as a catalyst [164].

Nevertheless, enzymatic transesterification still has its drawbacks. Among the main issues pertaining to the usage of an enzyme are the low stability and long reaction time, which lead to low production capacity. For example, an optimum reaction time of 56 h at 40 °C was required to produce 99.5% FAME conversion from *Nannochloropsis gaditana* using the lipase Novozym 435 of *Candida antarctica* as a catalyst with a lipase/oil mass ratio of 0.32 [165]. The authors also reported that the immobilised enzyme of the same batch could be used three times before the FAME conversion fell to 57%. This is due to the high content of polar lipids in microalgae, which resulted in the loss of enzyme activities [166]. Similar long hours are reported by other researchers using lipases as catalysts in the transesterification processes [164,167,168]. Another disadvantage of using biocatalyst is the high cost associated with them; for example, Novozym 435 costs around $9500/kg, which has become a big concern for scaling up [117]. However, quite recently, there are a few cheaper biocatalyst alternatives that can be used for commercial purposes [169]. A noteworthy comparative study among the reaction times taken by heterogeneous catalyst (tungstate zirconia), homogeneous catalyst (sulfuric acid), and biocatalyst (immobilised *Pseudomonas fluorescence* lipases) had been carried out recently by Guldhe et al. [170]. It was found that a shorter time was taken by heterogeneous catalyst compared to the homogeneous catalyst, while comparable FAME yields were obtained by the two methods. The heterogeneous catalyst also gave a higher yield compared to the biocatalyst. From the literature review, it can be inferred that heterogeneous catalysts have shown a better prospect to be used for in situ transesterification compared to homogenous catalysts and biocatalysts. Comparisons between different types of catalysts are given in Table 6 (adapted from [62,149]).

Table 6. Comparisons on the characteristics of conventional in situ transesterifications using different types of catalysts (adapted from [62,149]).

Type of Catalyst	% Yield	Advantages	Disadvantages
Homogeneous alkali	96–98	· fast reaction time · inexpensive · reaction happens at very mild condition	· suitable for biomass with low FFA (<2%)· difficult ester purification · catalyst cannot be recycled for the next usage · a large amount of water is needed for purification phase · excessive catalyst will lead to soap formation, hence making the purification step more difficult
Heterogeneous alkali	<90	· shorter reaction time than acid-catalyzed transesterification · catalysts can be separated and then reused · reaction happens at mild condition, and less energy is needed	· suitable for biomass with low FFA (<2%) · excessive catalyst will lead to soap formation, hence making the purification step more difficult · contamination of the final product might occur due to leaching · expensive synthesis method · long reaction time
Homogeneous acid	Up to 99	· suitable for biomass with high FFA and high moisture content· very suitable for in situ transesterification process · no saponification · inexpensive	· corrosive to the reactor and pipelines · difficult ester purification · excessive catalyst will lead to high acidity of product; hence requiring a lot of water for purification step · difficult to recover catalyst · high ratio of alcohol to oil is needed

Table 6. *Cont.*

Type of Catalyst	% Yield	Advantages	Disadvantages
Heterogonous acid	<90	· suitable for biomass with high FFA and high moisture content · very suitable for in situ transesterification process · no saponification · not corrosive to reactor and pipelines · catalysts can be separated and then reused	· long reaction time · expensive synthesis method in some cases · contamination of the final product might occur due to leaching · high ratio of alcohol to oil is needed
Biocatalyst	99	· suitable for biomass with high FFA and high moisture content · simple purification step · low ratio of alcohol to oil is needed · some are reusable (immobilised lipase) · reusable and inexpensive	· extremely long reaction time, slower than acid-catalyzed transesterification · generally very expensive · excessive methanol will lead to deactivation of biocatalyst
Carbon-based	<90	· simple synthesis method · high thermal stability · large surface area for more effective reaction	· long reaction time · leaching of SO_3H · high ratio of methanol to oil is needed

4.2. Advanced Methods for In Situ Transesterification

Apart from the conventional methods discussed above, there are advanced methods that utilise different solvents, technologies, or devices for biodiesel production via in situ transesterifications. Some are also combinations of several individual methods that are explicitly designed to simplify the processes or to obtain the highest possible yields during the execution of the in situ transesterification procedures.

4.2.1. Use of Co-Solvents

Co-solvents are sometimes used during an in situ transesterification to increase the reaction rate of the process due to the improvement of solubility between the microalgae's lipid and the alcohol used [117]. This is especially important because alcohol and water can mix to form a homogenous mixture and water is known to inhibit both extraction and transesterification of lipids. Therefore, more lipids can be extracted, and more mass transfer of the reactant can be obtained by the addition of a co-solvent during the process [64]. However, co-solvent does not take part in the reaction between the two. Hence the amount of co-solvent before and after the reaction would remain the same [171].

In terms of effectiveness, chloroform has a reputation as an excellent co-solvent for lipid extraction and in situ transesterification of microalgae biomass [130,172]. However, the characteristic of chloroform as a superior co-solvent is conditional, thereby depending on the nature of the methods used. In the work carried out by Najafabadi et al. [173] to investigate the effectiveness of several co-solvents for in situ transesterification of *Chlorella vulgaris* using supercritical methanol, it was reported that the highest FAME yield was obtained by using hexane as the co-solvent, with an optimum ratio of hexane to the biomass of 6 to 1. However, in the same study, the usage of chloroform actually resulted in less FAME yield than the one obtained by the control. It was concluded that for the case of in situ supercritical methanol transesterification, chloroform is considered to be a poor selection as a co-solvent.

Recently, water-immiscible ethyl acetate was used as a single reactant and co-solvent in a wet in situ transesterification of *Nannochloropsis gaditana* under acidic conditions [121]. The results were then compared to the ones obtained by using an ethanol/chloroform combination. As discussed above, alcohol is water-miscible, and the usage of alcohol alone during the reaction would reduce the effectiveness of the transesterification process. During the in situ transesterification process, ethyl acetate under acidic conditions is hydrolyzed into ethanol and acetic acid, wherein an ethanol/ethyl acetate binary phase is naturally

created [174]. The water inhibition effect can be minimized tremendously under this circumstance. In optimized conditions, an FAEE yield of 97.8% was obtained by using ethyl acetate, compared to around 77% by using an ethanol/chloroform combination. This further simplifies the in situ transesterification process since it can be carried out without much concern about the inhibiting effect of the microalgae's moisture content and the usage of hazardous chloroform.

The dependency on chloroform and other toxic co-solvents can be reduced by the utilisation of green solvents, such as ionic liquids. The usage of ionic liquids as co-solvents is considered to be a significant breakthrough in the production of biodiesel from microalgae, which has garnered tremendous interest from researchers and industries over the past 10 to 20 years [175]. This special class of liquid is considered an organic salt in liquid form at temperatures ranging from 0–140 °C, and its polarity can be changed by manipulating the anions and cations, hence it is applicable to a wide range of polar and non-polar compounds [176,177]. In addition, other attractive features of ionic liquids are their low vapor pressure, low volatility, low melting point, and variable viscosity [178]. The usage of ionic liquids has allowed in situ transesterifications of microalgae biomass to be done at lower temperatures and significantly less time compared to the conventional methods [179]. Ionic liquids are also well known for their ability to act as highly recyclable catalysts in the production of biodiesel from various feedstock [175].

In a study involving in situ transesterification using ionic liquid 1-butyl-3-methylimidazolium trifluoromethanesulfonate, [Bmim][CF_3SO_3] as a co-solvent, it was found that the FAME yield of wet *Nannochloropsis oceanica* under optimized conditions was 11-times higher than the yield from the control experiment, which utilised a two-step conversion using methanol and chloroform in the reaction [180]. When combined with other methods, such as microwave irradiation, the biodiesel yield using IL is proven to be satisfactory. In a study involving in situ transesterification using 1-ethyl-3-methylimidazolium methyl sulphate [EMIM][$MeSO_4$], a biodiesel yield of 40.9% was obtained when the wet *Nannochloropsis* sp. biomass-to-methanol (wt/vol %) ratio was set to be 1:4 and the methanol-to-IL (wt/vol %) ratio was set at 1:0.5 with a reaction time of 25 min [108]. However, in the same study, the biodiesel yield increased to 42.2% with a shorter reaction time of 14 min when the process was combined with microwave irradiation while keeping the microalgae-to-methanol and methanol-to-IL ratio constant. In a different study, ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate, [BMIm][PF_6] was mixed with immobilised lipase from *Penicillium expansum*, which acted as a catalyst [181]. From this study, it was found that under optimum conditions, a biodiesel yield of 90.7% was obtained from *Chlorella pyrenoidosa* with a reaction time of 48 h. These studies illustrated the versatility of ionic liquids in their application during in situ transesterifications of microalgae.

Another class of green solvents, called deep eutectic solvents, has gained much attention in the biodiesel production field for their use as co-solvents. These solvents are comprised of an organic salt that acts as a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD), thereby forming a eutectic mixture that has a lower melting point than each of the individual components [182,183]. Not only are they limited to disrupting the microalgae wall and to acting as catalysts, but deep eutectic solvents can also serve as a mild dehydrator that reduces the water content in the biomass, hence facilitating the transesterification process [184]. In addition to their non-toxicity and biodegradability, this new class of green solvents are superior to ionic liquids in terms of their purity, cost, and their easiness to prepare [185]. Since cheap and environmentally-friendly resources are used to prepare deep eutectic solvents, it is predicted that these would one day replace ionic liquids in the field of biodiesel production [186]. In a study, deep eutectic solvent Ch-Aa (which is comprised of choline chloride as the HBA and acetic acid as the HBD) was used for the in situ transesterification of wet microalgae *Chlorella* sp. and *Chlorococcum* sp. with 65–67% moisture contents [187]. It was found that the reaction carried out by using Ch-Aa gave a 30% higher total FAME yield compared to the yield from the conventional two-step esterification–transesterification method. This shows the effectiveness of deep

eutectic solvents in simplifying the process involved in the production of biodiesel. Even though the application of these types of solvents for in situ transesterification of microalgae is still considered as immature, deep eutectic solvents have shown great potential due to all the positive characteristics and the decent biodiesel yields.

Liquid polymers, such as polyethylene glycol and polypropylene glycols, have emerged as a new alternative solvent to be used in biodiesel productions. Although their usage in this area is considered relatively new, great potential has been shown by liquid polymers to be used as a co-solvent for in situ transesterification processes due to their attractive features, such as their eco-friendliness, non-volatility, and cost-effectiveness [188]. Recently, Liu et al. [189] evaluated the effectiveness of polyethylene glycol compared to chloroform in a rapid in situ transesterification of microalgae biomass using a minireactor. It was found that in addition to its capability to extract lipids, polyethylene glycol also acts as a suspension agent that facilitates the separation of FAME from the co-solvent. In the study, the effect of varying the molecular weight of liquid polymers was also examined, thereby leading to the conclusion that the properties of liquid polymers should be adjusted based on the needs of the experiment. Just like deep eutectic solvents, the advancement of liquid polymers to be utilised in biodiesel production is expected to rise significantly in the coming years [101].

4.2.2. Use of Microwave Assistance

In situ transesterification using the microwave is indeed favorable due to the fact that wet biomass can be utilised since the presence of water is actually beneficial for the process [190]. Among the main advantages of the microwave-assisted technique is the significant reduction of reaction time and energy consumption needed for the in situ transesterification process. In terms of energy usage, microwaves utilise only a third of the energy used in the conventional transesterification process [87]. Contrary to conventional techniques, which may take hours to be completed, the microwave-assisted technique has been proven to do the job effectively in just a few minutes. Using conventional transesterification, 98% of FAME recovery from microalgae biomass is possible with 20 h of reaction time, while 80% of FAME is achieved with 4 min of reaction time by microwave irradiation [191,192]. A higher FAEE yield is also obtained when using this method, compared to the conventional methods. In a comparative study between in situ transesterification via 6-min microwave irradiation and the conventional two-step Bligh and Dyer method, it was proven that the maximum FAEE conversion of *Chlorella* sp. lipid was 96.2% and 78.1%, respectively [107]. Due to the nature of non-contact heating possessed by microwave irradiation, the inside of the microalgae cell is heated more compared to conventional heating, in which the surface is subjected to more heat [193]. High temperatures and pressure build-up occurred within the microalgae cell as a result of the intermolecular fraction from the rapid heating, which leads to the rupture of the microalgae cell wall and the release of the intercellular lipid, as depicted in Figure 7 [87,194].

In a study, the wall of microalgae from the species *Chlorella* sp. was severely ruptured from exposure to microwave radiation, where the thickness of the cell was increased from 0.11 to 0.59 µm, and the pore diameter increased from 0.005 to 0.18 µm in a matter of minutes [195]. In a separate study, in situ transesterification was done under simultaneous cooling and microwave heating (SCMH), which allowed for better temperature control and more rigorous penetration from the irradiation, and resulted in almost a 5-times higher biodiesel production compared to other heating methods. Biodiesel yield from both the conventional water-bath heating method and microwave-assisted transesterification were then compared to the yield obtained from SCMH. The results were very encouraging, since the SCMH method produced 75% biodiesel, compared to 13.46% and 15.29% via the water bath and microwave method, respectively [196].

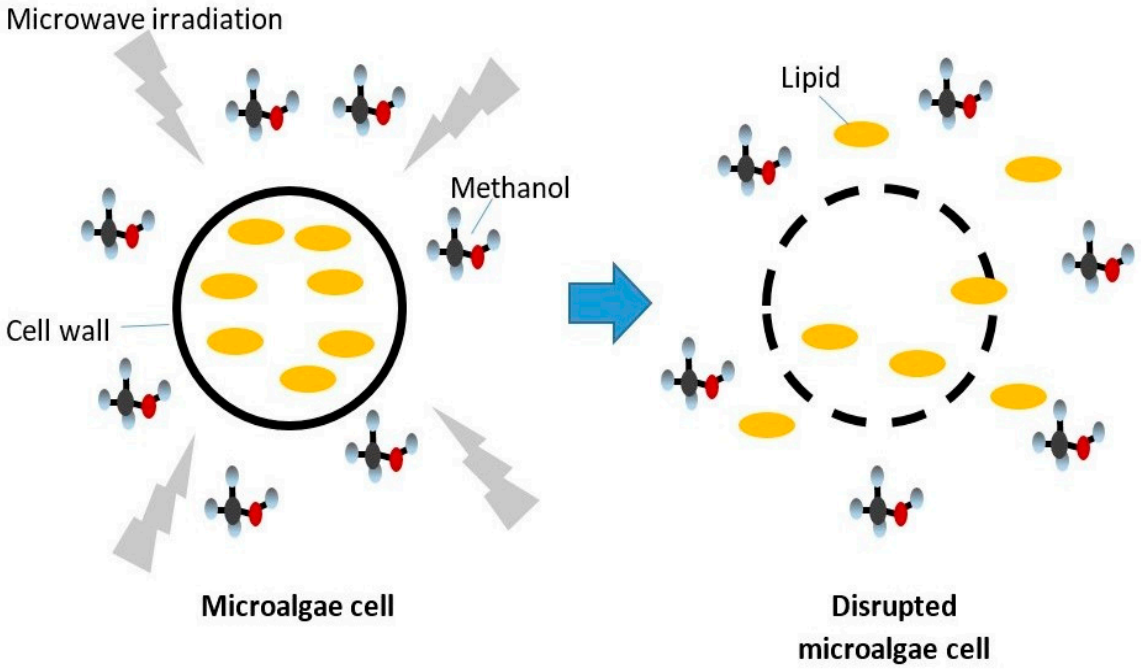

Figure 7. Microalgae cell disruption under microwave irradiation [81,132].

However, due to the rapid heating via microwave irradiation, non-uniform heating, which leads to the creation of hot spots, was observed to be one of the disadvantages of this method [197]. For the commercial production of biodiesel using the microwave, among the main concerns are the scalability of the microwave device and the ability of the method to penetrate a large amount of feedstock at a time, since the penetration depth of microwaves is reported to only be between 10 to 20 mm [198,199]. From the literature review, even though microwave-assisted in situ transesterification has been proven to work effectively in lab-scale studies, more in-depth research needs to be done before its implementation for commercial-scale biodiesel production.

4.2.3. Use of Ultrasound Assistance

The ultrasound method involves the generation of sound waves that create alternate high and low pressures through the fluid [200]. The set-up of an ultrasonic device for the in situ transesterification experiment involving microalgae is shown in Figure 8 (adapted from [201]). Cavitation occurs when small bubbles that were created during the low-pressure cycle suddenly burst during the high-pressure cycle. The cellular walls of the biomass are then ruptured due to the shear force created during the cavitation event. Ultrasound is an effective method to increase the mass transfer between the immiscible liquid phases [202]. The disruption of the oil–alcohol boundary from the ultrasound agitation leads to an intense mixing of the compounds and faster formation of alkyl ester, thus improving the yield and shortening the reaction times [203]. A study was conducted to evaluate the effectiveness of the ultrasound method for in situ transesterifications [132]. Without ultrasound, the lipid extraction amounted to only 12.8%, leading to a 63.6% ester content after 2 h of reaction time. When the same process was repeated using ultrasound set at 80 W, a 97.6% ester content was obtained while the lipid yield was almost unchanged. When the intensity of the ultrasound was set to be at 180 W, the lipid yield and ester content increased to 26% and 96.9%, respectively. This further demonstrated the effectiveness of the ultrasound-assisted in situ transesterification method.

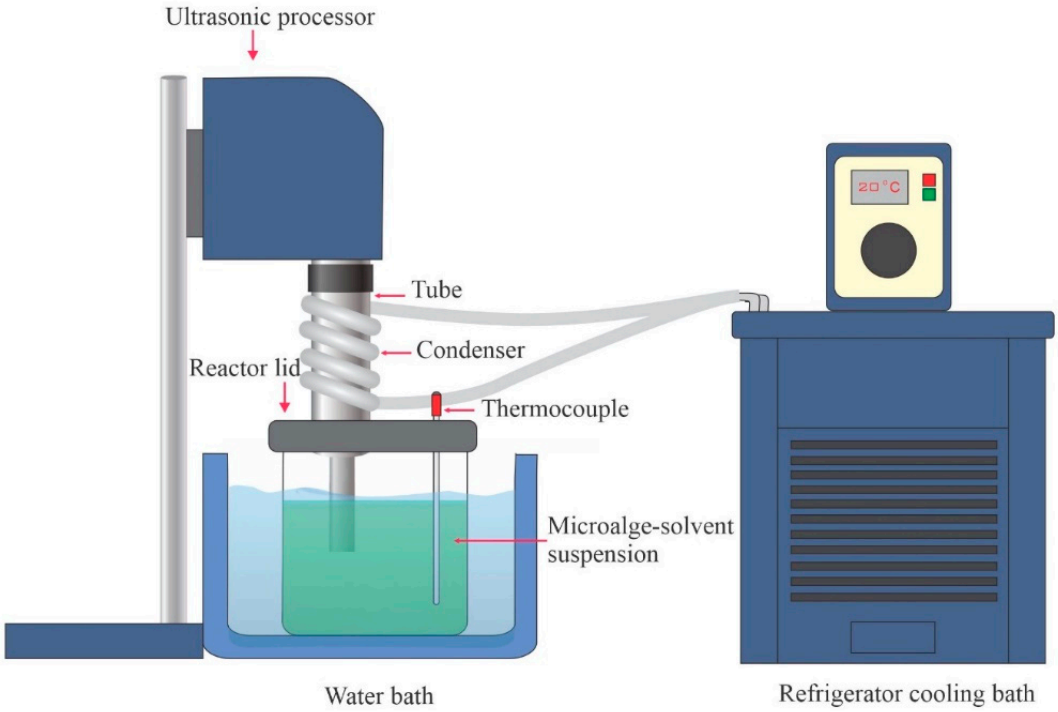

Figure 8. Schematic diagram for ultrasonic assisted in situ transesterification (adapted from [201]).

In a comparative study between the effectiveness of the microwave and ultrasound method during in situ transesterification, it was found that the microwave method was able to produce better biodiesel and FAEE yields at a lower power level, while the ultrasound method was superior in producing higher yields at lower solvent ratios [204]. In the same study, it was calculated that the energy required for the microwave-assisted method and ultrasound methods are 26 MJ/kg and 44 MJ/kg of biodiesel, respectively. In a recent in situ transesterification study conducted by Cercado et al. [205], an 85% biodiesel yield was obtained from *Chlorella vulgaris* via the ultrasound method. In the process, a novel alkali catalyst called K-pumice was used, and the results were very encouraging since it was done by setting the alcohol-to-lipid molar ratio to 12:1 with a reaction time of 10 min, which is a significant improvement when compared to the conventional in situ transesterification process.

4.2.4. Synergistic Microwave and Ultrasound Assistance

There have been some studies done to investigate the suitability of using a synergistic in situ transesterification system consisting of microwave- and ultrasound-assisted methods. As discussed above, the microwave-assisted method has the ability to rapidly heat the microalgae biomass, which is something that is not possessed by the ultrasound-assisted method. However, the ultrasound-assisted method has the mass transferability, which is a characteristic that a microwave-assisted method is lacking. Thus, theoretically, by combining these two methods, cell walls can be ruptured, the mass transfer can be enhanced, and the reaction rate can be further improved [206].

Several works have been carried out recently to evaluate the effect of combining these two methods. A comparative study between three methods—synergistic microwave with the ultrasound-assisted method, individual microwave-assisted method, and the individual ultrasound-assisted method—was done by Ma et al. [207]. In the study, it was discovered that a maximum FAME yield of 93.07 $\pm$ 2.39% was obtained from the in situ transesterification process using the microwave–ultrasound reactor, compared to only 63.49 $\pm$ 4.69% and 58.12 $\pm$ 2.84% from the individual ultrasound-assisted method and microwave-assisted method, respectively. This proves that the synergy of these two methods significantly improves the FAME yield. In a recent work by Martinez-Guerra et al. [208], the determination

of optimum conditions for the synergistic method was conducted using response surface methodology. It was found that 140W of microwave power and 140 W of ultrasound power were required to have a maximum FAME yield of 48.2% for a wet *Nannochloropsis* sp. biomass-to-methanol ratio of 20 g to 30 mL and 1 wt % NaOH catalyst concentration with a 7-min reaction time. This shows that relatively low powers of microwave and ultrasound are needed to produce a satisfactory FAME yield.

4.2.5. Supercritical Fluid Conditions

In recent years, the usage of supercritical fluids has attracted a lot of interest from researchers as an alternative to the traditional extraction process [209]. In the field of biodiesel production, supercritical fluid conditions can be applied to in situ transesterification to facilitate the rupture of microalgae walls and, consequently, to convert the lipid into biodiesel in one step, thereby reducing the energy consumption and increasing the final yield [92,210,211]. This method can also be combined with other methods, such as ultrasound, to enhance the final yield further or to simplify the process [210]. Figure 9 shows the schematic diagram of a supercritical reaction unit using supercritical carbon dioxide as a co-solvent in the production of biodiesel from *Spirulina platensis* microalga [212].

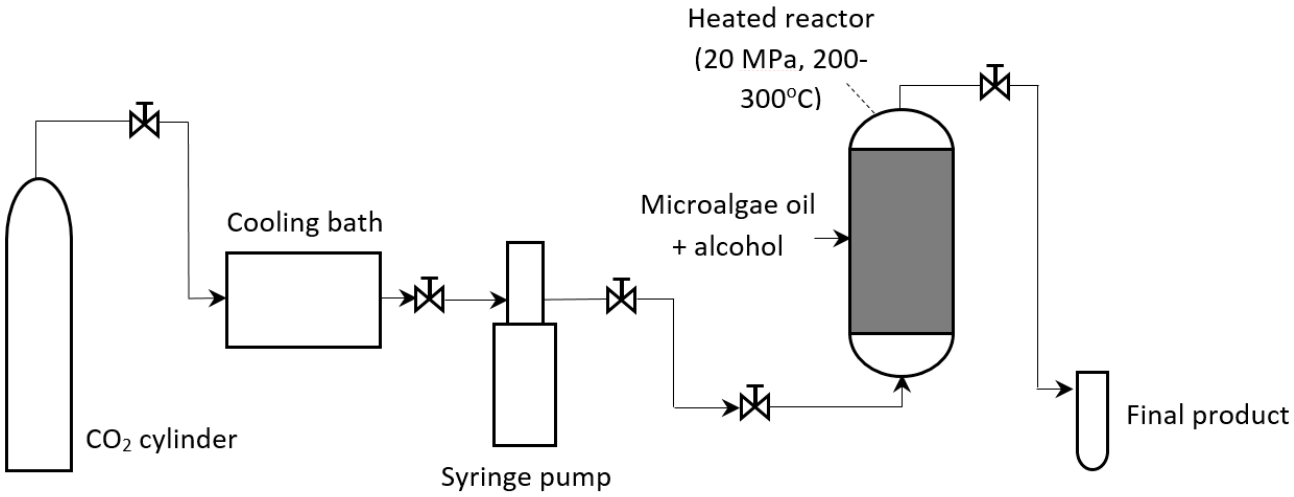

Figure 9. Schematic diagram of a supercritical reaction unit in the production of biodiesel from microalgae [212].

Jafari et al. [213] studied the in situ transesterification of wet *Nannochloropsis oculata* impregnated with 15 cc ethanol per 1 g dry microalgae to produce biodiesel using supercritical carbon dioxide (CO_2). They showed that the production efficiency of biodiesel was affected by the temperature and water content, with a significant deteriorative effect on productivity at temperatures below 150 °C. They also reported a yield of 24.3 wt. % at about 150 °C with biomass containing 80% water. In another study, the production of biodiesel from wet *Nannochloropsis salina* using supercritical ethanol was studied to measure the effect of reaction time, reaction temperature, and the ratio of biomass to ethanol on overall FAEE in order to find the optimum operating conditions [214]. It was found that a 67% FAEE yield was obtained for 20-min reaction time at the temperature of 265 °C using a biomass-to-ethanol ratio (wt./vol.) of 1 to 9. There was also investigative work done using supercritical methanol for the transesterification process involving microalgae. Response surface methodology combined with central composite design was employed in the in situ transesterification of *Spirulina platensis* using supercritical methanol to evaluate the optimum conditions of various parameters to the overall FAME yield [215]. From the experiments, a FAME yield of 99.32% was obtained using temperature, reaction time, the ratio of methanol to dry algae, the ratio of co-solvent (hexane) to dry algae, moisture

content, critical temperature, and critical pressure of 300 °C, 30 min, 8 to 1, 4 to 1, 40%, 244.8 °C, and 6.61 MPa, respectively.

In a comparative study between microwave-assisted and non-catalytic supercritical methanol in situ transesterification, it was proven that the former would result in a better yield at a shorter reaction time, while the latter would produce a more purified final product [216]. In other words, even though non-catalytic supercritical methanol generally takes a longer reaction time and a lesser yield compared to the microwave-assisted method, less energy and time will be spent during the purification and separation phases, since the final product is free from the catalyst and environmentally-unfriendly solvent residues. Recently, an attempt was made to use supercritical CO_2 to extract lipids from *Spirulina platensis* at a large pilot scale using biomass ranging from 1 kg to 50 kg [217]. It was proven that lipid extraction using supercritical CO_2 is suitable even at such a huge amount of biomass. However, the work only covered the lipid extraction of the biomass until the transesterification process. Nevertheless, this could be a good indicator to prove the feasibility of implementing in situ transesterification using supercritical CO_2 for large-scale biodiesel production.

4.2.6. Hydrothermal Liquefaction

Hydrothermal liquefaction is a method considered to perfectly suit liquid biofuel production from wet microalgae biomass even without the presence of catalysts since the method utilises water at supercritical or subcritical conditions, which act as a green solvent [218]. Although this method seems to work very well due to the high-water content in the freshly-harvested microalgae, the reaction needs to happen under extreme conditions at above 300 °C and 10 MPa [211].

However, most of the hydrothermal liquefaction works involving microalgae have been done up to the stage where biocrude was produced, not until the stage where biodiesel is directly obtained after the process [96,219]. The produced biocrude still needs to undergo a separation process for obtaining biodiesel. Kim et al. [220] conducted a study by combining the in situ transesterification process with hydrothermal liquefaction to produce FAEE from wet *Nannochloropsis gaditana* microalgae using several solvents. In addition to lipids, the product of hydrothermal liquefaction is biocrude, which contains other non-lipid substances like protein, cellulose, and pigments. Hence, it is very crucial to separate the FAEE to be used for producing biodiesel. In the study, it was found that dichloroethane has the highest FAEE selectivity in the produced biocrude, yielding 91.85% of the maximum transesterifiable lipid. The usage of this particular solvent would facilitate the production of FAEE without much effort in the separation and purification phase.

Several life cycle analyses have been done on this method, and based on the currently available technologies, it is reported that hydrothermal liquefaction is the best option to produce biofuel from microalgae on a commercial scale [221–223]. Furthermore, integration of microalgae cultivation and microalgae hydrothermal liquefaction processes could increase the biomass up to 10-times [224]. So far, there is still plenty of improvements that need to be done for in situ transesterification using the hydrothermal liquefaction method.

5. Perspectives and Future Directions

In situ transesterification technology is progressing continuously under various experimental conditions using a different type of biomass; however, it has not been fully developed for an industrial scale due to the technical hurdles [225,226]. Among the main issues pertaining to the biodiesel production from microalgae are cost, scalability, and its eventual impact on the environment. To compete with fossil fuels, research needs to be conducted continuously, especially on the processing aspects. Modifications and improvements, even at the smallest scale, need to be done meticulously starting from the upstream in order to have a significant total reduction in the overall production cost. There is an aura of optimism surrounding the realization of commercial microalgal biofuel in the future due to the advantages and advancements of in situ transesterification in recent years.

Even though the implementation of in situ transesterification managed to circumvent the separated drying and lipid extraction from microalgae, a huge amount of heat is needed in order to carry out the process, which posed a big challenge in scaling up. Combinations of various methods that lead to a lower reaction temperature would mean a lot in the effort for commercialization.

The microalgae biofuel bubble experienced by some companies in recent years has made many people have skepticism about the potential and advantages possessed by microalgae. Their closures have left a negative perception among the people about the feasibility of microalgae as a viable feedstock for biofuel. This cannot be the benchmark in the effort to develop and expand the knowledge in producing biodiesel from microalgae. It is said that most of the failures resulted from a lack of knowledge in microalgae biology [227]. This is primarily driven by the eagerness of the business sectors to join the bandwagon after hearing the potential of microalgae as a source of green fuel. However, due to immature technologies and procedures, they were forced to cease operations after not seeing profits within their stipulated timeframes. Techno-economic analyses done by researchers can be used to evaluate the feasibility of a microalgae biodiesel plant to convince the industries and the policymakers to further invest and support the microalgae industry. For this, a biorefinery approach that utilises in situ transesterification could be a wise option during the scale-up phase for commercialization purposes. When in situ transesterification is coupled with this concept, other high-value byproducts related to the nutritional, pharmaceuticals, and cosmetics industries, such as pigments, glycerol, antioxidants, and health food supplements, can be derived in the effort to further reduce or offset the high cost associated with biodiesel production [228,229]. During the design stage, the system should also offer some sort of unique solutions for the existing environmental issues faced by the community at the locations of interest, for example, carbon dioxide sequestration from the power plant and wastewater treatment from industries.

Due to the vast selections of species and the ability to practically grow in any part of the world, microalgae serve as the most promising biodiesel feedstock. More species of microalgae need to be explored to evaluate their suitability as feedstock for biodiesel production. Somehow, types of microalgae from the diatom class have been mostly overlooked, even though they are regarded as one of the most promising feedstocks for biofuel production by the US Energy Department [230]. Even though these classes of eukaryotic microalgae are excellent at accumulating lipids (especially under starvation of silica), only a handful of species have been explored for biofuel production purposes [231,232]. By understanding the biology of diatoms and subsequently utilising the in situ transesterification process on these high lipid content species, more options will be available in the effort to diversify the pathways for biodiesel productions.

Further improvement needs to be made on the usage of inexpensive green solvents for the in situ transesterification of microalgae. The utilisation of these environmentally-friendly substances is considered a huge step in spurring the positive growth in the microalgae-based biodiesel industry. More renewable and clean energy supporters can be obtained by enhancing the biodiesel procedures using green solvents since fewer negative impacts are imposed on Mother Nature throughout the process. Moreover, rigorous studies related to the combination of in situ transesterification and hydrothermal liquefaction of microalgae biomass should be further explored. As mentioned in Section 4.2.6, hydrothermal liquefaction has been identified as one of the most promising methods for the realization of microalgae-based biodiesel commercialization. With the right paradigms and approaches, microalgae could one day be globally used to partly power the world.

6. Conclusions

Various methods related to recent practices for the in situ transesterification of microalgae biomass have been reviewed. Among the main concerns are the cost, the energy consumption of the methods, and the environmental impact as a whole. Looking at these aspects, the usage of green solvents and effective methods with low energy requirements

are among the recommended pathways for future explorations. Some methods have great potential for scaling-up purposes, while others are only suitable for lab-scale studies. It has been shown that in situ transesterification is the most effective way to produce biodiesel from microalgae. In low temperatures, physical assistance (microwave, ultrasound etc.) and the use of co-solvents (ionic liquid, hexane, chloroform) have been used alone or concurrently to accelerate the simultaneous lipid extraction and transesterification. At high temperatures, usually above 150 °C, the modified characteristics of water and alcohol at their sub/supercritical state makes the process simple by getting rid of the need for catalysts and co-solvents. However, an in situ transesterification method with superior characteristics may contain flaws when it is compared to other methods, and vice versa. Nevertheless, a combination of in situ transesterification and hydrothermal liquefaction is found to be one of the most promising methods in simplifying the processes involved in producing biodiesel from microalgae. Not only can the energy extensive dewatering and lipid extraction stages be circumvented, but the usage of a catalyst can also be avoided via this technique. Furthermore, a special class of algae called diatoms should be extensively studied to further maximize the potential of biodiesel production from this organism. With more in-depth studies on the combinations of methods and continuous explorations for novel techniques, commercialized microalgae-based biodiesel could be a reality in the future.

Author Contributions: Conceptualization writing original draft preparation; F.I., Writing-review & editing—Supporting; M.F.M.A.Z., Funding acquisition-Lead, Methodology-Equal, Project administration-Lead, Supervision-Lead, Writing—review & editing-Lead; A.H.S. and S.N., Investigation-Equal, Methodology-Equal, Resources-Equal; F.K., Data curation-Equal, Resources-Equal, Writing—review & editing-Equal; I.M.R.F. and Project administration-Lead, Supervision-Lead, Writing—review & editing-Lead; T.M.I.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Ministry of Energy and Natural Resources [AAIBE Chair for Renewable Energy grant number KETTHA 202003].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors would like to acknowledge Ministry of Energy and Natural Resources and UNITEN for supporting the research facilities. Fazril Ideris would also like to thank UNITEN for the BOLD Postgraduate Excellence Scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. IEA. *World Energy Outlook 2017*; IEA: Paris, France, 2017.
2. Zhou, Z.; Slaný, M.; Kuzielová, E.; Zhang, W.; Ma, L.; Dong, S.; Zhang, J.; Chen, G. Influence of Reservoir Minerals and Ethanol on Catalytic Aquathermolysis of Heavy Oil. *Fuel* **2022**, *307*, 121871. [CrossRef]
3. Chiari, L.; Zecca, A. Constraints of Fossil Fuels Depletion on Global Warming Projections. *Energy Policy* **2011**, *39*, 5026–5034. [CrossRef]
4. Ideris, F.; Shamsuddin, A.H.; Nomanbhay, S.; Kusumo, F.; Silitonga, A.S.; Ong, M.Y.; Ong, H.C.; Mahlia, T.M.I. Optimization of Ultrasound-Assisted Oil Extraction from Canarium Odontophyllum Kernel as a Novel Biodiesel Feedstock. *J. Clean. Prod.* **2021**, *288*, 125563. [CrossRef]

5. Khairudin, N.F.; Bidin, R.; Shamsuddin, A.H.; Akhiar, A.; Ideris, F.; Abd Rahman, A. Renewable Energy Development in Malaysia: Communication Barriers towards Achieving the National Renewable Energy Target. In Proceedings of the 2nd International Conference on Civil & Environmental Engineering, Langkawi, Malaysia, 20–21 November 2019; IOP Conference Series: Earth and Environmental Science; Volume 476. [CrossRef]
6. Masjuki, H.H.; Kalam, M.A.; Mofijur, M.; Shahabuddin, M. Biofuel: Policy, Standardization and Recommendation for Sustainable Future Energy Supply. *Energy Procedia* **2013**, *42*, 577–586. [CrossRef]
7. Kusumo, F.; Shamsuddin, A.H.; Ahmad, A.R.; Dharma, S.; Milano, J.; Silitonga, A.S.; Fazril, I.; Marzuki, H.; Akhiar, A.; Sebayang, R.; et al. Production of Biodiesel from Jatropha Curcas Mixed with Waste Cooking Oil Assisted by Ultrasound. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *476*, 012082. [CrossRef]
8. Silitonga, A.S.; Masjuki, H.H.; Ong, H.C.; Sebayang, A.H.; Dharma, S.; Kusumo, F.; Siswantoro, J.; Milano, J.; Daud, K.; Mahlia, T.M.I.; et al. Evaluation of the Engine Performance and Exhaust Emissions of Biodiesel-Bioethanol-Diesel Blends Using Kernel-Based Extreme Learning Machine. *Energy* **2018**, *159*, 1075–1087. [CrossRef]
9. Atabani, A.; Silitonga, A.; Ong, H. Non-Edible Vegetable Oils: A Critical Evaluation of Oil Extraction, Fatty Acid Compositions, Biodiesel Production, Characteristics, Engine Performance and Emissions. *Sustain. Energy* **2013**, *18*, 211–245. [CrossRef]
10. Imtenan, S.; Varman, M.; Masjuki, H.H.; Kalam, M.A.; Sajjad, H.; Arbab, M.I.; Rizwanul Fattah, I.M. Impact of Low Temperature Combustion Attaining Strategies on Diesel Engine Emissions for Diesel and Biodiesels: A Review. *Energy Convers. Manag.* **2014**, *80*, 329–356. [CrossRef]
11. Marzuki, H.; Shamsuddin, A.H.; Saifuddin, N.M.; Ideris, F. Energy Saving Potential Using Elite Jatropha Curcas Hybrid for Biodiesel Production in Malaysia. *Int. J. Recent Technol. Eng.* **2019**, *8*, 6281–6287. [CrossRef]
12. Hafriz, R.S.R.M.; Arifin, N.A.; Salmiaton, A.; Yunus, R.; Taufiq-Yap, Y.H.; Saifuddin, N.M.; Shamsuddin, A.H. Multiple-Objective Optimization in Green Fuel Production via Catalytic Deoxygenation Reaction with NiO-Dolomite Catalyst. *Fuel* **2022**, *308*, 122041. [CrossRef]
13. Lane, J. Biofuels Digest. Available online: https://www.biofuelsdigest.com/bdigest/2015/09/10/methanols-moment-with-low-prices-and-proven-process-is-meth-your-new-best-friend/ (accessed on 5 June 2021).
14. Phongamwong, T.; Chantaprasertporn, U.; Witoon, T.; Numpilai, T.; Poo-arporn, Y.; Limphirat, W.; Donphai, W.; Dittanet, P.; Chareonpanich, M.; Limtrakul, J. CO_2 Hydrogenation to Methanol over CuO–ZnO–ZrO_2–SiO_2 Catalysts: Effects of SiO_2 Contents. *Chem. Eng. J.* **2017**, *316*, 692–703. [CrossRef]
15. Greenhalgh, K. Methanol Production Capacity May Quintuple on Decarbonized Industry Transformation: Study | IHS Markit. Available online: https://cleanenergynews.ihsmarkit.com/research-analysis/methanol-production-capacity-may-quintuple-on-decarbonized-ind.html (accessed on 22 September 2022).
16. Babarit, A.; Body, E.; Gilloteaux, J.C.; Hetet, J.F. Energy and Economic Performance of the FARWIND Energy System for Sustainable Fuel Production from the Far-Offshore Wind Energy Resource. In Proceedings of the Fourteenth International Conference on Ecological Vehicles and Renewable Energies (EVER), Monte-Carlo, Monaco, 8–10 May 2019; pp. 1–10. [CrossRef]
17. Borisut, P.; Nuchitprasittichai, A. Methanol Production via CO_2 Hydrogenation: Sensitivity Analysis and Simulation—Based Optimization. *Front. Energy Res.* **2019**, *7*, 81. [CrossRef]
18. Bos, M.J.; Kersten, S.R.A.; Brilman, D.W.F. Wind Power to Methanol: Renewable Methanol Production Using Electricity, Electrolysis of Water and CO_2 Air Capture. *Appl. Energy* **2020**, *264*, 114672. [CrossRef]
19. von der Assen, N.; Jung, J.; Bardow, A. Life-Cycle Assessment of Carbon Dioxide Capture and Utilization: Avoiding the Pitfalls. *Energy Environ. Sci.* **2013**, *6*, 2721–2734. [CrossRef]
20. Karmakar, R.; Kundu, K.; Rajor, A. Fuel Properties and Emission Characteristics of Biodiesel Produced from Unused Algae Grown in India. *Pet. Sci.* **2018**, *15*, 385–395. [CrossRef]
21. Sugözü, İ.; Öner, C.; Altun, Ş. The Performance and Emissions Characteristics of a Diesel Engine Fueled with Biodiesel and Diesel Fuel. *Int. J. Eng. Res.* **2010**, *2*, 50–53.
22. James, G.; Speight, K.S. Fuels From Biomass. In *Environmental Management of Energy from Biofuels and Biofeedstocks*; Wiley: Hoboken, NY, USA, 2014; Web; pp. 1–51. ISBN 9781118915141.
23. Huang, Q.; Jiang, F.; Wang, L.; Yang, C. Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms. *Engineering* **2017**, *3*, 318–329. [CrossRef]
24. Im, H.; Kim, B.; Lee, J.W. Bioresource Technology Concurrent Production of Biodiesel and Chemicals through Wet in Situ Transesterification of Microalgae. *Bioresour. Technol.* **2015**, *193*, 386–392. [CrossRef]
25. Ong, H.C.; Masjuki, H.H.; Mahlia, T.M.I.; Silitonga, A.S.; Chong, W.T.; Leong, K.Y. Optimization of Biodiesel Production and Engine Performance from High Free Fatty Acid Calophyllum Inophyllum Oil in CI Diesel Engine. *Energy Convers. Manag.* **2014**, *81*, 30–40. [CrossRef]
26. Bagchi, S.K.; Rao, P.S.; Mallick, N. Bioresource Technology Development of an Oven Drying Protocol to Improve Biodiesel Production for an Indigenous Chlorophycean *Microalga scenedesmus* sp. *Bioresour. Technol.* **2015**, *180*, 207–213. [CrossRef]
27. Ma, Y.; Gao, Z.; Wang, Q.; Liu, Y. Bioresource Technology Biodiesels from Microbial Oils: Opportunity and Challenges. *Bioresour. Technol.* **2018**, *263*, 631–641. [CrossRef] [PubMed]
28. Salam, K.A.; Velasquez-orta, S.B.; Harvey, A.P. A Sustainable Integrated in Situ Transesteri Fi Cation of Microalgae for Biodiesel Production and Associated Co-Product-a Review. *Renew. Sustain. Energy Rev.* **2016**, *65*, 1179–1198. [CrossRef]

29. Kim, B.; Young, H.; Son, J.; Yang, J.; Chang, Y.; Lee, J.H.; Lee, J.W. Simplifying Biodiesel Production from Microalgae via Wet in Situ Transesteri Fi Cation: A Review in Current Research and Future Prospects. *Algal Res.* **2019**, *41*, 101557. [CrossRef]
30. Park, J.; Park, M.S.; Lee, Y.; Yang, J. Bioresource Technology Advances in Direct Transesterification of Algal Oils from Wet Biomass. *Bioresour. Technol.* **2015**, *184*, 267–275. [CrossRef] [PubMed]
31. Iderisa, F.; Susan Silitonga, A.; Kusumo, F.; Halim Shamsuddina, A.; Nomanbhaya, S.M.; Sebayang, A.H.; Sutrisno, J.; Bela, N. The Effect of Ultrasound Duty Cycle in Biodiesel Production from Ceiba Pentandra. *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *753*, 012031. [CrossRef]
32. Kusumo, F.; Mahlia, T.M.I.; Shamsuddin, A.H.; Ahmad, A.R.; Silitonga, A.S.; Dharma, S.; Mofijur, M.; Ideris, F.; Ong, H.C.; Sebayang, R.; et al. Optimisation of Biodiesel Production from Mixed Sterculia Foetida and Rice Bran Oil. *Int. J. Ambient Energy* **2021**, 1–11. [CrossRef]
33. Andreo-Martínez, P.; Ortiz-Martínez, V.M.; Salar-García, M.J.; Veiga-del-Baño, J.M.; Chica, A.; Quesada-Medina, J. Waste Animal Fats as Feedstock for Biodiesel Production Using Non-Catalytic Supercritical Alcohol Transesterification: A Perspective by the PRISMA Methodology. *Energy Sustain. Dev.* **2022**, *69*, 150–163. [CrossRef]
34. Godwin John, J.; Hariram, V.; Kavuru Rakesh, V.S.S.; Harsha Vardhan, T.; Vamsi Manikanta, T.Y.; Shafi, S. Waste Cooking Oil Biodiesel with FeO Nanoparticle–A Viable Alternative Fuel Source. *Mater. Today Proc.* 2022; *in press*. [CrossRef]
35. Soudagar, M.E.M.; Nik-Ghazali, N.-N.; Kalam, M.A.; Badruddin, I.A.; Banapurmath, N.R.; Yunus Khan, T.M.; Bashir, M.N.; Akram, N.; Farade, R.; Afzal, A. The Effects of Graphene Oxide Nanoparticle Additive Stably Dispersed in Dairy Scum Oil Biodiesel-Diesel Fuel Blend on CI Engine: Performance, Emission and Combustion Characteristics. *Fuel* **2019**, *257*, 116015. [CrossRef]
36. Fazril, I.; Shamsuddin, A.H.; Nomanbhay, S.; Kusomo, F.; Hanif, M.; Ahmad Zamri, M.F.M.; Akhiar, A.; Ismail, M.F. Microwave-Assisted in Situ Transesterification of Wet Microalgae for the Production of Biodiesel: Progress Review. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *476*, 12078. [CrossRef]
37. Mahlia, T.M.I.; Syazmi, Z.A.H.S.; Mofijur, M.; Abas, A.E.P.; Bilad, M.R.; Ong, H.C.; Silitonga, A.S. Patent Landscape Review on Biodiesel Production: Technology Updates. *Renew. Sustain. Energy Rev.* **2020**, *118*, 109526. [CrossRef]
38. Silitonga, A.S.; Shamsuddin, A.H.; Mahlia, T.M.I.; Milano, J.; Kusumo, F.; Siswantoro, J.; Dharma, S.; Sebayang, A.H.; Masjuki, H.H.; Ong, H.C. Biodiesel Synthesis from Ceiba Pentandra Oil by Microwave Irradiation-Assisted Transesterification: ELM Modeling and Optimization. *Renew. Energy* **2020**, *146*, 1278–1291. [CrossRef]
39. Moser, B.R. Biodiesel Production, Properties, and Feedstocks. *Vitr. Cell. Dev. Biol.-Plant* **2009**, *45*, 229–266. [CrossRef]
40. Mofijur, M.; Atabani, A.E.; Masjuki, H.H.; Kalam, M.A.; Masum, B.M. A Study on the Effects of Promising Edible and Non-Edible Biodiesel Feedstocks on Engine Performance and Emissions Production: A Comparative Evaluation. *Renew. Sustain. Energy Rev.* **2013**, *23*, 391–404. [CrossRef]
41. Han, B.; Goh, H.; Chyuan, H.; Yee, M.; Chen, W.; Ling, K. Sustainability of Direct Biodiesel Synthesis from Microalgae Biomass: A Critical Review. *Renew. Sustain. Energy Rev.* **2019**, *107*, 59–74. [CrossRef]
42. Mubarak, M.; Shaija, A.; Suchithra, T.V. Review Article A Review on the Extraction of Lipid from Microalgae for Biodiesel Production. *Algal* **2015**, *7*, 117–123. [CrossRef]
43. Demirbas, M.F. Biofuels from Algae for Sustainable Development. *Appl. Energy* **2011**, *88*, 3473–3480. [CrossRef]
44. Martins, A.; Caetano, N.S.; Mata, T.M. Microalgae for Biodiesel Production and Other Applications: A Review. *Renew. Sustain. Energy Rev.* **2010**, *14*, 217–232. [CrossRef]
45. Silveira Junior, E.; Simionatto, E.; Perez, V.; Justo, O.; Zárate, N.; Vieira, M. Potential of Virginia-Type Peanut (*Arachis hypogaea* L.) as Feedstock for Biodiesel Production. *Ind. Crops Prod.* **2016**, *89*, 448–454. [CrossRef]
46. Moser, B. Preparation of Fatty Acid Methyl Esters from Hazelnut, High-Oleic Peanut and Walnut Oils and Evaluation as Biodiesel. *Fuel* **2012**, *92*, 231–238. [CrossRef]
47. Chisti, Y. Biodiesel from Microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306. [CrossRef] [PubMed]
48. Hanifzadeh, M.; Sarrafzadeh, M.-H.; Nabati, Z.; Tavakoli, O.; Feyzizarnagh, H. Technical, Economic and Energy Assessment of an Alternative Strategy for Mass Production of Biomass and Lipid from Microalgae. *J. Environ. Chem. Eng.* **2018**, *6*, 866–873. [CrossRef]
49. Suganya, T.; Varman, M.; Masjuki, H.H.; Renganathan, S. Macroalgae and Microalgae as a Potential Source for Commercial Applications along with Biofuels Production: A Biore Fi Nery Approach. *Renew. Sustain. Energy Rev.* **2016**, *55*, 909–941. [CrossRef]
50. Nadiah, W.; Kadir, A.; Kee, M.; Uemura, Y.; Wei, J.; Teong, K. Harvesting and Pre-Treatment of Microalgae Cultivated in Wastewater for Biodiesel Production: A Review. *Energy Convers. Manag.* **2018**, *171*, 1416–1429. [CrossRef]
51. Hanif, M.; Hesam, N.M.; Akhiar, A.; Fazril, I.; Zamri, M.F.M.A.; Shamsuddin, A.H. Economic Feasibility of Smart City Power Generation from Biogas Produced by Food Waste in Malaysia via Techno-Economic Analysis. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *476*, 012076. [CrossRef]
52. Park, J.B.K.; Craggs, R.J.; Shilton, A.N. Bioresource Technology Wastewater Treatment High Rate Algal Ponds for Biofuel Production. *Bioresour. Technol.* **2011**, *102*, 35–42. [CrossRef] [PubMed]
53. Li, J.; Otero-Gonzalez, L.; Michiels, J.; Lens, P.N.L.; Du Laing, G.; Ferrer, I. Production of Selenium-Enriched Microalgae as Potential Feed Supplement in High-Rate Algae Ponds Treating Domestic Wastewater. *Bioresour. Technol.* **2021**, *333*, 125239. [CrossRef]

54. Passos, F.; Ferrer, I. Microalgae Conversion to Biogas: Thermal Pretreatment Contribution on Net Energy Production. *Environ. Sci. Technol.* **2014**, *48*, 7171–7178. [CrossRef] [PubMed]
55. Wahlen, B.D.; Willis, R.M.; Seefeldt, L.C. Biodiesel Production by Simultaneous Extraction and Conversion of Total Lipids from Microalgae, Cyanobacteria, and Wild Mixed-Cultures. *Bioresour. Technol.* **2011**, *102*, 2724–2730. [CrossRef]
56. Dasgupta, C.N.; Suseela, M.R.; Mandotra, S.K.; Kumar, P.; Pandey, M.K.; Toppo, K.; Lone, J.A. Dual Uses of Microalgal Biomass: An Integrative Approach for Biohydrogen and Biodiesel Production. *Appl. Energy* **2015**, *146*, 202–208. [CrossRef]
57. Wan, C.; Bai, F.-W.; Zhao, X.-Q. Effects of Nitrogen Concentration and Media Replacement on Cell Growth and Lipid Production of Oleaginous Marine Microalga Nannochloropsis Oceanica DUT01. *Biochem. Eng. J.* **2013**, *78*, 32–38. [CrossRef]
58. Islam, M.A.; Magnusson, M.; Brown, R.J.; Ayoko, G.A.; Nabi, M.N.; Heimann, K. Microalgal Species Selection for Biodiesel Production Based on Fuel Properties Derived from Fatty Acid Profiles. *Energies* **2013**, *6*, 5676–5702. [CrossRef]
59. Ho, S.; Lai, Y.; Chiang, C.; Chen, C.N.; Chang, J. Bioresource Technology Selection of Elite Microalgae for Biodiesel Production in Tropical Conditions Using a Standardized Platform. *Bioresour. Technol.* **2013**, *147*, 135–142. [CrossRef] [PubMed]
60. Gouveia, L.; Cristina, A. Microalgae as a Raw Material for Biofuels Production. *J. Ind. Microbiol. Biotechnol.* **2009**, *142*, 269–274. [CrossRef]
61. Khan, S.; Siddique, R.; Sajjad, W.; Nabi, G.; Hayat, K.M.; Duan, P.; Yao, L. Biodiesel Production From Algae to Overcome the Energy Crisis. *Hayati J. Biosci.* **2017**, *24*, 163–167. [CrossRef]
62. Zhu, L.; Nugroho, Y.K.; Shakeel, S.R.; Li, Z.; Martinkauppi, B.; Hiltunen, E. Using Microalgae to Produce Liquid Transportation Biodiesel: What Is Next? *Renew. Sustain. Energy Rev.* **2017**, *78*, 391–400. [CrossRef]
63. Hidalgo, P.; Ciudad, G.; Schober, S.; Mittelbach, M.; Navia, R. Improving the FAME Yield of in Situ Transesterification from Microalgal Biomass through Particle Size Reduction and Cosolvent Incorporation. *Energy Fuels* **2015**, *29*, 823–832. [CrossRef]
64. Zhang, Y.; Li, Y.; Zhang, X.; Tan, T. Bioresource Technology Biodiesel Production by Direct Transesterification of Microalgal Biomass with Co-Solvent. *Bioresour. Technol.* **2015**, *196*, 712–715. [CrossRef]
65. Chen, J.; Li, J.; Dong, W.; Zhang, X.; Tyagi, R.D.; Drogui, P.; Surampalli, R.Y. The Potential of Microalgae in Biodiesel Production. *Renew. Sustain. Energy Rev.* **2018**, *90*, 336–346. [CrossRef]
66. Shwetharani, R.; Balakrishna, R.G. Efficient Algal Lipid Extraction via Photocatalysis and Its Conversion to Biofuel. *Appl. Energy* **2016**, *168*, 364–374. [CrossRef]
67. Ehimen, E.A.; Sun, Z.F.; Carrington, C.G. Variables Affecting the in Situ Transesterification of Microalgae Lipids. *Fuel* **2010**, *89*, 677–684. [CrossRef]
68. Halim, R.; Danquah, M.K.; Webley, P.A. Extraction of Oil from Microalgae for Biodiesel Production: A Review. *Biotechnol. Adv.* **2012**, *30*, 709–732. [CrossRef] [PubMed]
69. Ghasemi Naghdi, F.; González González, L.M.; Chan, W.; Schenk, P.M. Progress on Lipid Extraction from Wet Algal Biomass for Biodiesel Production. *Microb. Biotechnol.* **2016**, *9*, 718–726. [CrossRef]
70. Ríos, S.D.; Castañeda, J.; Torras, C.; Farriol, X.; Salvadó, J. Bioresource Technology Lipid Extraction Methods from Microalgal Biomass Harvested by Two Different Paths: Screening Studies toward Biodiesel Production. *Bioresour. Technol.* **2013**, *133*, 378–388. [CrossRef] [PubMed]
71. Ratledge, C.; Cohen, Z. Microbial and Algal Oils: Do They Have a Future for Biodiesel or as Commodity Oils? *Lipid Technol. Wiley* **2008**, *20*, 155–160. [CrossRef]
72. Reijnders, L. Do Biofuels from Microalgae Beat Biofuels from Terrestrial Plants? *Trends Biotechnol.* **2008**, *1*, 15–16. [CrossRef] [PubMed]
73. Taylor, P.; Hannon, M.; Gimpel, J.; Tran, M.; Rasala, B.; Mayfield, S. Biofuels from Algae: Challenges and Potential Biofuels from Algae: Challenges and Potential. *Biofuels* **2014**, *1*, 37–41. [CrossRef]
74. Chen, C.; Huang, C.; Ho, K.; Hsiao, P.; Wu, M. Bioresource Technology Biodiesel Production from Wet Microalgae Feedstock Using Sequential Wet Extraction/Transesterification and Direct Transesterification Processes. *Bioresour. Technol.* **2015**, *194*, 179–186. [CrossRef]
75. Phong, W.N.; Show, P.L.; Ling, T.C.; Juan, J.C.; Ng, E.-P.; Chang, J.-S. Mild Cell Disruption Methods for Bio-Functional Proteins Recovery from Microalgae—Recent Developments and Future Perspectives. *Algal Res.* **2018**, *31*, 506–516. [CrossRef]
76. Lee, S.Y.; Cho, J.M.; Chang, Y.K.; Oh, Y.-K. Cell Disruption and Lipid Extraction for Microalgal Biorefineries: A Review. *Bioresour. Technol.* **2017**, *244*, 1317–1328. [CrossRef]
77. Li, T.; Zheng, Y.; Yu, L.; Chen, S. Mixotrophic Cultivation of a Chlorella Sorokiniana Strain for Enhanced Biomass and Lipid Production. *Biomass Bioenergy* **2014**, *66*, 204–213. [CrossRef]
78. Rashidi, B.; Trindade, L.M. Detailed Biochemical and Morphologic Characteristics of the Green Microalga Neochloris Oleoabundans Cell Wall. *Algal Res.* **2018**, *35*, 152–159. [CrossRef]
79. Guiry, M.D. P Erspective How Many Species of Algae Are There? *J. Phycol.* **2012**, *48*, 1057–1063. [CrossRef]
80. Gerken, H.G.; Donohoe, B.; Knoshaug, E.P. Enzymatic Cell Wall Degradation of Chlorella Vulgaris and Other Microalgae for Biofuels Production. *Planta* **2013**, *237*, 239–253. [CrossRef]
81. Adair, W.S.; Steinmetz, S.A.; Mattson, D.M.; Goodenough, U.W.; Heuser, J.E. Chlamydomonas Volvox. *J. Cell Biol.* **1987**, *105*, 2373–2382. [CrossRef] [PubMed]
82. Al-Muhteseb, S.; Emeish, S. Producing Natural Mixed Carotenoids from Dunaliella Salina. *J. Nat. Sci. Res.* **2015**, *5*, 53–59.

83. Praveenkumar, R.; Lee, K.; Lee, J.; Oh, Y.-K. Breaking Dormancy: An Energy-Efficient Means of Recovering Astaxanthin from Microalgae. *Green Chem.* **2015**, *17*, 1226–1234. [CrossRef]
84. Scholz, M.J.; Weiss, T.L.; Jinkerson, R.E.; Jing, J.; Roth, R.; Posewitz, M.C.; Gerken, H.G.; Biology, C.; State, A. Ultrastructure and Composition of the Nannochloropsis Gaditana Cell Wall. *Eukaryot. Cell* **2014**, *13*, 1450–1464. [CrossRef] [PubMed]
85. Lu, W.; Alam, A.; Pan, Y.; Wu, J.; Wang, Z.; Yuan, Z. Bioresource Technology A New Approach of Microalgal Biomass Pretreatment Using Deep Eutectic Solvents for Enhanced Lipid Recovery for Biodiesel Production. *Bioresour. Technol.* **2016**, *218*, 123–128. [CrossRef]
86. Halim, R.; Rupasinghe, T.W.T.; Tull, D.L.; Webley, P.A. Bioresource Technology Mechanical Cell Disruption for Lipid Extraction from Microalgal Biomass. *Bioresour. Technol.* **2013**, *140*, 53–63. [CrossRef]
87. Wahidin, S.; Idris, A.; Raehanah, S.; Shaleh, M. Rapid Biodiesel Production Using Wet Microalgae via Microwave Irradiation. *Energy Convers. Manag.* **2014**, *84*, 227–233. [CrossRef]
88. Günerken, E.; Hondt, E.D.; Eppink, M.H.M.; Garcia-gonzalez, L.; Elst, K.; Wijffels, R.H. Cell Disruption for Microalgae Biore Fi Neries. *Biotechnol. Adv.* **2015**, *33*, 243–260. [CrossRef] [PubMed]
89. Show, K.; Lee, D.; Tay, J.; Lee, T.; Chang, J. Bioresource Technology Microalgal Drying and Cell Disruption–Recent Advances. *Bioresour. Technol.* **2015**, *184*, 258–266. [CrossRef] [PubMed]
90. Greenwell, H.C.; Laurens, L.M.L.; Shields, R.J.; Lovitt, R.W.; Flynn, K.J. Placing Microalgae on the Biofuels Priority List: A Review of the Technological Challenges Placing Microalgae on the Biofuels Priority List: A Review of the Technological Challenges. *J. R. Soc. Interface* **2010**, 703–726. [CrossRef] [PubMed]
91. Shirgaonkar, I.Z.; Lothe, R.R.; Pandit, A.B. Comments on the Mechanism of Microbial Cell Disruption in High-Pressure and High-Speed Devices. *Biotechnol. Prog.* **1998**, *14*, 657–660. [CrossRef]
92. Joannes, C.; Sipaut, C.S.; Dayou, J.; Yasir, S.; Mansa, R.F. The Potential of Using Pulsed Electric Field (PEF) Technology as the Cell Disruption Method to Extract Lipid from Microalgae for Biodiesel Production. *Int. J. Renew. Energy Res.* **2015**, *5*, 598–621.
93. Zhang, Y.; Soldatov, S.; Papachristou, I.; Nazarova, N.; Link, G.; Frey, W.; Silve, A. Pulsed Microwave Pretreatment of Fresh Microalgae for Enhanced Lipid Extraction. *Energy* **2022**, *248*, 123555. [CrossRef]
94. Zheng, H.; Yin, J.; Gao, Z.; Huang, H. Disruption of Chlorella Vulgaris Cells for the Release of Biodiesel-Producing Lipids: A Comparison of Grinding, Ultrasonication, Bead Milling, Enzymatic Lysis, and Microwaves. *Appl. Biochem. Biotechnol.* **2011**, *167*, 1215–1224. [CrossRef]
95. Luengo, E.; Martínez, J.M.; Bordetas, A.; Álvarez, I.; Raso, J. Influence of the Treatment Medium Temperature on Lutein Extraction Assisted by Pulsed Electric Fields from Chlorella Vulgaris. *Innov. Food Sci. Emerg. Technol.* **2015**, *29*, 15–22. [CrossRef]
96. Jazrawi, C.; Biller, P.; He, Y.; Montoya, A.; Ross, A.B.; Maschmeyer, T.; Haynes, B.S. Two-Stage Hydrothermal Liquefaction of a High-Protein Microalga. *Algal* **2015**, *8*, 15–22. [CrossRef]
97. Zhang, J.; Xia, A.; Chen, H.; Nizami, A.-S.; Huang, Y.; Zhu, X.; Zhu, X.; Liao, Q. Biobased Carbon Dots Production via Hydrothermal Conversion of Microalgae Chlorella Pyrenoidosa. *Sci. Total Environ.* **2022**, *839*, 156144. [CrossRef]
98. Grossmann, L.; Ebert, S.; Hinrichs, J.; Weiss, J. Effect of Precipitation, Lyophilization, and Organic Solvent Extraction on Preparation of Protein-Rich Powders from the Microalgae Chlorella Protothecoides. *Algal Res.* **2018**, *29*, 266–276. [CrossRef]
99. Halim, R.; Gladman, B.; Danquah, M.K.; Webley, P.A. Bioresource Technology Oil Extraction from Microalgae for Biodiesel Production. *Bioresour. Technol.* **2011**, *102*, 178–185. [CrossRef] [PubMed]
100. Tang, W.; Ho Row, K. Evaluation of CO_2-Induced Azole-Based Switchable Ionic Liquid with Hydrophobic/Hydrophilic Reversible Transition as Single Solvent System for Coupling Lipid Extraction and Separation from Wet Microalgae. *Bioresour. Technol.* **2020**, *296*, 122309. [CrossRef] [PubMed]
101. Jeevan Kumar, S.P.; Vijay Kumar, G.; Dash, A.; Scholz, P.; Banerjee, R. Sustainable Green Solvents and Techniques for Lipid Extraction from Microalgae: A Review. *Algal Res.* **2017**, *21*, 138–147. [CrossRef]
102. Seo, J.Y.; Praveenkumar, R.; Kim, B.; Seo, J.-C.; Park, J.-Y.; Na, J.-G.; Jeon, S.G.; Park, S.B.; Lee, K.; Oh, Y.-K. Downstream Integration of Microalgae Harvesting and Cell Disruption by Means of Cationic Surfactant-Decorated Fe_3O_4 Nanoparticles. *Green Chem.* **2016**, *18*, 3981–3989. [CrossRef]
103. Taylor, P.; Huang, Y.; Hong, A.; Zhang, D.; Li, L. Comparison of Cell Rupturing by Ozonation and Ultrasonication for Algal Lipid Extraction from Chlorella Vulgaris. *Environ. Technol.* **2014**, *35*, 931–937. [CrossRef]
104. Krishna Koyande, A.; Tanzil, V.; Murraly Dharan, H.; Subramaniam, M.; Robert, R.N.; Lau, P.-L.; Khoiroh, I.; Show, P.-L. Integration of Osmotic Shock Assisted Liquid Biphasic System for Protein Extraction from Microalgae Chlorella Vulgaris. *Biochem. Eng. J.* **2020**, *157*, 107532. [CrossRef]
105. Zinnai, A.; Sanmartin, C.; Taglieri, I.; Andrich, G.; Venturi, F. Supercritical Fluid Extraction from Microalgae with High Content of LC-PUFAs. A Case of Study: Sc-CO_2 Oil Extraction from *Schizochytrium* sp. *J. Supercrit. Fluids* **2016**, *116*, 126–131. [CrossRef]
106. Sierra, L.S.; Dixon, C.K.; Wilken, L.R. Enzymatic Cell Disruption of the Microalgae Chlamydomonas Reinhardtii for Lipid and Protein Extraction. *Algal Res.* **2017**, *25*, 149–159. [CrossRef]
107. Martinez-guerra, E.; Gnaneswar, V.; Mondala, A.; Holmes, W.; Hernandez, R. Bioresource Technology Extractive-Transesterification of Algal Lipids under Microwave Irradiation with Hexane as Solvent. *Bioresour. Technol.* **2014**, *156*, 240–247. [CrossRef]
108. Wahidin, S.; Idris, A.; Mohd, N.; Hisham, N.; Kamis, H. Optimization of the Ionic Liquid-Microwave Assisted One-Step Biodiesel Production Process from Wet Microalgal Biomass. *Energy Convers. Manag.* **2018**, *171*, 1397–1404. [CrossRef]

109. Adeniyi, O.M.; Azimov, U.; Burluka, A. Algae Biofuel: Current Status and Future Applications. *Renew. Sustain. Energy Rev.* **2018**, *90*, 316–335. [CrossRef]
110. Boey, P.-L.; Ganesan, S.; Maniam, G.P.; Ali, D.M.H. Ultrasound Aided in Situ Transesterification of Crude Palm Oil Adsorbed on Spent Bleaching Clay. *Energy Convers. Manag.* **2011**, *52*, 2081–2084. [CrossRef]
111. Yu, G.-W.; Nie, J.; Lu, L.-G.; Wang, S.-P.; Li, Z.-G.; Lee, M.-R. Transesterification of Soybean Oil by Using the Synergistic Microwave-Ultrasonic Irradiation. *Ultrason. Sonochem.* **2017**, *39*, 281–290. [CrossRef] [PubMed]
112. Martínez, A.; Mijangos, G.E.; Romero-Ibarra, I.C.; Hernández-Altamirano, R.; Mena-Cervantes, V.Y. In-Situ Transesterification of Jatropha Curcas L. Seeds Using Homogeneous and Heterogeneous Basic Catalysts. *Fuel* **2019**, *235*, 277–287. [CrossRef]
113. Dasari, S.R.; Chaudhary, A.J.; Goud, V.V.; Sahoo, N.; Kulkarni, V.N. In-Situ Alkaline Transesterification of Castor Seeds: Optimization and Engine Performance, Combustion and Emission Characteristics of Blends. *Energy Convers. Manag.* **2017**, *142*, 200–214. [CrossRef]
114. Koutsouki, A.A.; Tegou, E.; Badeka, A.; Kontakos, S.; Pomonis, P.J.; Kontominas, M.G. In Situ and Conventional Transesterification of Rapeseeds for Biodiesel Production: The Effect of Direct Sonication. *Ind. Crops Prod.* **2016**, *84*, 399–407. [CrossRef]
115. Taherkhani, M.; Sadrameli, S.M. An Improvement and Optimization Study of Biodiesel Production from Linseed via In-Situ Transesterification Using a Co-Solvent. *Renew. Energy* **2018**, *119*, 787–794. [CrossRef]
116. Yellapu, S.K.; Kaur, R.; Tyagi, R.D. Detergent Assisted Ultrasonication Aided in Situ Transesterification for Biodiesel Production from Oleaginous Yeast Wet Biomass. *Bioresour. Technol.* **2017**, *224*, 365–372. [CrossRef]
117. Woo, A.; Sutanto, S.; Ki, L.; Tran-nguyen, P.L.; Ismadji, S.; Ju, Y. Developments in In-Situ (Trans) Esteri Fi Cation for Biodiesel Production: A Critical Review. *Renew. Sustain. Energy Rev.* **2016**, *60*, 284–305. [CrossRef]
118. Eguchi, S.; Kagawa, S.; Okamoto, S. Environmental and Economic Performance of a Biodiesel Plant Using Waste Cooking Oil. *J. Clean. Prod.* **2015**, *101*, 245–250. [CrossRef]
119. Fulton, L.; Mejia, A.; Arioli, M.; Dematera, K.; Lah, O. Climate Change Mitigation Pathways for Southeast Asia: CO_2 Emissions Reduction Policies for the Energy and Transport Sectors. *Sustainability* **2017**, *9*, 1160. [CrossRef]
120. Vandyck, T.; Keramidas, K.; Saveyn, B.; Kitous, A.; Vrontisi, Z. A Global Stocktake of the Paris Pledges: Implications for Energy Systems and Economy. *Glob. Environ. Chang.* **2016**, *41*, 46–63. [CrossRef]
121. Park, J.; Kim, B.; Keun, Y.; Lee, J.W. Bioresource Technology Wet in Situ Transesterification of Microalgae Using Ethyl Acetate as a Co-Solvent and Reactant. *Bioresour. Technol.* **2017**, *230*, 8–14. [CrossRef] [PubMed]
122. Pradana, Y.S.; Sudibyo, H.; Suyono, E.A.; Indarto; Budiman, A. Oil Algae Extraction of Selected Microalgae Species Grown in Monoculture and Mixed Cultures for Biodiesel Production. *Energy Procedia* **2017**, *105*, 277–282. [CrossRef]
123. Johnson, M.B.; Wen, Z. Production of Biodiesel Fuel from the Microalga Schizochytrium Limacinum by Direct Transesterification of Algal Biomass. *Energy Fuels* **2009**, *48*, 5179–5183. [CrossRef]
124. Harrington, K.J.; D'Arcy-Evans, C. Transesterification in Situ of Sunflower Seed Oil. *Ind. Eng. Chem. Prod. Res. Dev.* **1985**, *24*, 314–318. [CrossRef]
125. Sivaramakrishnan, R.; Incharoensakdi, A. Bioresource Technology Microalgae as Feedstock for Biodiesel Production under Ultrasound Treatment–A Review. *Bioresour. Technol.* **2018**, *250*, 877–887. [CrossRef]
126. Hidalgo, P.; Ciudad, G.; Mittelbach, M.; Navia, R. Biodiesel Production by Direct Conversion of Botryococcus Braunii Lipids: Reaction Kinetics Modelling and Optimization. *Fuel* **2015**, *153*, 544–551. [CrossRef]
127. Kim, B.; Im, H.; Lee, J.W. Bioresource Technology In Situ Transesterification of Highly Wet Microalgae Using Hydrochloric Acid. *Bioresour. Technol.* **2015**, *185*, 421–425. [CrossRef] [PubMed]
128. Lemões, J.S.; Alves, R.C.M.; Farias, S.P.; De Moura, R.R.; Primel, E.G.; Abreu, P.C.; Martins, A.F.; Montes, M.G.; Oca, D. Sustainable Production of Biodiesel from Microalgae by Direct Transesteri Fi Cation. *Sustain. Chem. Pharm.* **2016**, *3*, 33–38. [CrossRef]
129. Vonortas, A.; Papayannakos, N. Comparative Analysis of Biodiesel versus Green Diesel. *Wiley Interdiscip. Rev. Energy Environ.* **2014**, *3*, 3–23. [CrossRef]
130. Im, H.; Lee, H.; Park, M.S.; Yang, J.; Lee, J.W. Bioresource Technology Concurrent Extraction and Reaction for the Production of Biodiesel from Wet Microalgae. *Bioresour. Technol.* **2014**, *152*, 534–537. [CrossRef] [PubMed]
131. Tran, D.-T.; Yeh, K.-L.; Chen, C.-L.; Chang, J.-S. Enzymatic Transesterification of Microalgal Oil from Chlorella Vulgaris ESP-31 for Biodiesel Synthesis Using Immobilized Burkholderia Lipase. *Bioresour. Technol.* **2012**, *108*, 119–127. [CrossRef] [PubMed]
132. Martínez, N.; Callejas, N.; Morais, E.G.; Vieira Costa, J.A.; Jachmanián, I.; Vieitez, I. Obtaining Biodiesel from Microalgae Oil Using Ultrasound-Assisted in-Situ Alkaline Transesterification. *Fuel* **2017**, *202*, 512–519. [CrossRef]
133. Salam, K.A.; Velasquez-orta, S.B.; Harvey, A.P. Kinetics of Fast Alkali Reactive Extraction/in Situ Transesteri Fi Cation of Chlorella Vulgaris That Identi Fi Es Process Conditions for a Signi Fi Cant Enhanced Rate and Water Tolerance. *Fuel Process. Technol.* **2016**, *144*, 212–219. [CrossRef]
134. Leung, D.Y.C.; Wu, X.; Leung, M.K.H. A Review on Biodiesel Production Using Catalyzed Transesterification. *Appl. Energy* **2010**, *87*, 1083–1095. [CrossRef]
135. Velasquez-Orta, S.B.; Lee, J.G.M.; Harvey, A. Alkaline in Situ Transesterification of Chlorella Vulgaris. *Fuel* **2012**, *94*, 544–550. [CrossRef]
136. Thliveros, P.; Uçkun Kiran, E.; Webb, C. Microbial Biodiesel Production by Direct Methanolysis of Oleaginous Biomass. *Bioresour. Technol.* **2014**, *157*, 181–187. [CrossRef] [PubMed]

137. Sani, Y.M.; Daud, W.M.A.W.; Abdul Aziz, A.R. Solid Acid-Catalyzed Biodiesel Production from Microalgal Oil—The Dual Advantage. *J. Environ. Chem. Eng.* **2013**, *1*, 113–121. [CrossRef]
138. Galadima, A.; Muraza, O. Biodiesel Production from Algae by Using Heterogeneous Catalysts: A Critical Review. *Energy* **2014**, *78*, 72–83. [CrossRef]
139. Vicente, G.; Carrero, A.; Rodríguez, R.; Peso, G.L. Heterogeneous-Catalysed Direct Transformation of Microalga Biomass into Biodiesel-Grade FAMEs. *Fuel* **2017**, *200*, 590–598. [CrossRef]
140. Galadima, A.; Muraza, O. Hydrothermal Liquefaction of Algae and Bio-Oil Upgrading into Liquid Fuels: Role of Heterogeneous Catalysts. *Renew. Sustain. Energy Rev.* **2018**, *81*, 1037–1048. [CrossRef]
141. Cheng, J.; Qiu, Y.; Huang, R.; Yang, W.; Zhou, J.; Cen, K. Bioresource Technology Biodiesel Production from Wet Microalgae by Using Graphene Oxide as Solid Acid Catalyst. *Bioresour. Technol.* **2016**, *221*, 344–349. [CrossRef] [PubMed]
142. Mansir, N.; Taufiq-Yap, Y.H.; Rashid, U.; Lokman, I.M. Investigation of Heterogeneous Solid Acid Catalyst Performance on Low Grade Feedstocks for Biodiesel Production: A Review. *Energy Convers. Manag.* **2017**, *141*, 171–182. [CrossRef]
143. Skorupskaite, V.; Makareviciene, V.; Gumbyte, M. Opportunities for Simultaneous Oil Extraction and Transesteri Fi Cation during Biodiesel Fuel Production from Microalgae: A Review. *Fuel Process. Technol.* **2016**, *150*, 78–87. [CrossRef]
144. Guldhe, A.; Singh, B.; Rawat, I.; Bux, F. Synthesis of Biodiesel from Scenedesmus Sp. by Microwave and Ultrasound Assisted in Situ Transesterification Using Tungstated Zirconia as a Solid Acid Catalyst. *Chem. Eng. Res. Des.* **2014**, *92*, 1503–1511. [CrossRef]
145. Choo, M.; Eng, L.; Loke, P.; Chang, J.; Chuan, T. Journal of the Taiwan Institute of Chemical Engineers Recent Progress in Catalytic Conversion of Microalgae Oil to Green Hydrocarbon: A Review. *J. Taiwan Inst. Chem. Eng.* **2017**, *79*, 116–124. [CrossRef]
146. Malins, K.; Kampars, V.; Brinks, J.; Neibolte, I.; Murnieks, R. Synthesis of Activated Carbon Based Heterogenous Acid Catalyst for Biodiesel Preparation. *Appl. Catal. B Environ.* **2015**, *176–177*, 553–558. [CrossRef]
147. Jeon, J.L.T. A Review of the Terahertz Conductivity of Bulk and Nano-Materials. *J. Infrared Millim. Waves* **2012**, *33*, 871–925. [CrossRef]
148. Konwar, L.J.; Wärnå, J.; Mäki-Arvela, P.; Kumar, N.; Mikkola, J.-P. Reaction Kinetics with Catalyst Deactivation in Simultaneous Esterification and Transesterification of Acid Oils to Biodiesel (FAME) over a Mesoporous Sulphonated Carbon Catalyst. *Fuel* **2016**, *166*, 1–11. [CrossRef]
149. Konwar, L.J.; Boro, J.; Deka, D. Review on Latest Developments in Biodiesel Production Using Carbon-Based Catalysts. *Renew. Sustain. Energy Rev.* **2014**, *29*, 546–564. [CrossRef]
150. Cheng, J.; Qiu, Y.; Zhang, J.; Huang, R.; Yang, W.; Fan, Z. Bioresource Technology Conversion of Lipids from Wet Microalgae into Biodiesel Using Sulfonated Graphene Oxide Catalysts. *Bioresour. Technol.* **2017**, *244*, 569–574. [CrossRef] [PubMed]
151. Ferreira Mota, G.; Germano de Sousa, I.; Luiz Barros de Oliveira, A.; Luthierre Gama Cavalcante, A.; da Silva Moreira, K.; Thálysson Tavares Cavalcante, F.; Erick da Silva Souza, J.; Rafael de Aguiar Falcão, Í.; Guimarães Rocha, T.; Bussons Rodrigues Valério, R.; et al. Biodiesel Production from Microalgae Using Lipase-Based Catalysts: Current Challenges and Prospects. *Algal Res.* **2022**, *62*, 102616. [CrossRef]
152. Hama, S.; Kondo, A. Enzymatic Biodiesel Production: An Overview of Potential Feedstocks and Process Development. *Bioresour. Technol.* **2013**, *135*, 386–395. [CrossRef]
153. Guldhe, A.; Singh, P.; Kumari, S.; Rawat, I.; Permaul, K.; Bux, F. Biodiesel Synthesis from Microalgae Using Immobilized Aspergillus Niger Whole Cell Lipase Biocatalyst. *Renew. Energy* **2016**, *85*, 1002–1010. [CrossRef]
154. Tian, X.; Dai, L.; Liu, M.; Liu, D.; Du, W.; Wu, H. Lipase-Catalyzed Methanolysis of Microalgae Oil for Biodiesel Production and PUFAs Concentration. *Catal. Commun.* **2016**, *84*, 44–47. [CrossRef]
155. Teo, C.L.; Jamaluddin, H.; Zain, N.A.M.; Idris, A. Biodiesel Production via Lipase Catalysed Transesterification of Microalgae Lipids from *Tetraselmis* Sp. *Renew. Energy* **2014**, *68*, 1–5. [CrossRef]
156. Sivaramakrishnan, R.; Muthukumar, K. Direct Transesterification of Oedogonium Sp. Oil Be Using Immobilized Isolated Novel Bacillus Sp. Lipase. *J. Biosci. Bioeng.* **2014**, *117*, 86–91. [CrossRef] [PubMed]
157. He, Y.; Wu, T.; Wang, X.; Chen, B.; Chen, F. Bioresource Technology Cost-e Ff Ective Biodiesel Production from Wet Microalgal Biomass by a Novel Two-Step Enzymatic Process. *Bioresour. Technol.* **2018**, *268*, 583–591. [CrossRef]
158. Raou, Z.; Latif, S.; Gargari, M. Biodiesel Production from Microalgae Oil by Lipase from Pseudomonas Aeruginosa Displayed on Yeast Cell Surface. *Biochem. Eng. J.* **2018**, *140*, 1–8. [CrossRef]
159. Bayramoglu, G.; Akbulut, A.; Ozalp, V.C.; Arica, M.Y. Immobilized Lipase on Micro-Porous Biosilica for Enzymatic Transesterification of Algal Oil. *Chem. Eng. Res. Des.* **2015**, *95*, 12–21. [CrossRef]
160. Sivaramakrishnan, R.; Incharoensakdi, A. Direct Transesterification of *Botryococcus* sp. Catalysed by Immobilized Lipase: Ultrasound Treatment Can Reduce Reaction Time with High Yield of Methyl Ester. *Fuel* **2017**, *191*, 363–370. [CrossRef]
161. Huang, J.; Xia, J.; Jiang, W.; Li, Y.; Li, J. Biodiesel Production from Microalgae Oil Catalyzed by a Recombinant Lipase. *Bioresour. Technol.* **2015**, *180*, 47–53. [CrossRef]
162. Kim, K.H.; Lee, O.K.; Kim, C.H.; Seo, J.-W.; Oh, B.-R.; Lee, E.Y. Lipase-Catalyzed in-Situ Biosynthesis of Glycerol-Free Biodiesel from Heterotrophic Microalgae, Aurantiochytrium Sp. KRS101 Biomass. *Bioresour. Technol.* **2016**, *211*, 472–477. [CrossRef]
163. Wu, S.; Song, L.; Sommerfeld, M.; Hu, Q.; Chen, W. Optimization of an Effective Method for the Conversion of Crude Algal Lipids into Biodiesel. *Fuel* **2017**, *197*, 467–473. [CrossRef]

164. Bautista, L.F.; Vicente, G.; Mendoza, A.; Gonza, S.; Morales, V.; Rey, U.; Carlos, J.; Tulipa, C. Enzymatic Production of Biodiesel from Nannochloropsis Gaditana Microalgae Using Immobilized Lipases in Mesoporous Materials. *Energy Fuels* **2015**, *29*, 4981–8989. [CrossRef]
165. Navarro López, E.; Robles Medina, A.; Esteban Cerdán, L.; González Moreno, P.A.; Macías Sánchez, M.D.; Molina Grima, E. Fatty Acid Methyl Ester Production from Wet Microalgal Biomass by Lipase-Catalyzed Direct Transesterification. *Biomass Bioenergy* **2016**, *93*, 6–12. [CrossRef]
166. Amoah, J.; Ho, S.-H.; Hama, S.; Yoshida, A.; Nakanishi, A.; Hasunuma, T.; Ogino, C.; Kondo, A. Lipase Cocktail for Efficient Conversion of Oils Containing Phospholipids to Biodiesel. *Bioresour. Technol.* **2016**, *211*, 224–230. [CrossRef]
167. Jin, Z.; Han, S.; Zhang, L.; Zheng, S.; Wang, Y.; Lin, Y. Bioresource Technology Combined Utilization of Lipase-Displaying Pichia Pastoris Whole-Cell Biocatalysts to Improve Biodiesel Production in Co-Solvent Media. *Bioresour. Technol.* **2013**, *130*, 102–109. [CrossRef]
168. Sivaramakrishnan, R.; Incharoensakdi, A. Purification and Characterization of Solvent Tolerant Lipase from *Bacillus* Sp. for Methyl Ester Production from Algal Oil. *J. Biosci. Bioeng.* **2016**, *121*, 517–522. [CrossRef] [PubMed]
169. Price, J.; Nordblad, M.; Martel, H.H.; Chrabas, B.; Wang, H.; Nielsen, P.M.; Woodley, J.M.; Llc, V.F. Scale-Up of Industrial Biodiesel Production to 40 m 3 Using a Liquid Lipase Formulation. *Biotechnol. Bioeng.* **2016**, *113*, 1719–1728. [CrossRef]
170. Guldhe, A.; Singh, P.; Ahmad, F.; Singh, B.; Bux, F. Biodiesel Synthesis from Microalgal Lipids Using Tungstated Zirconia as a Heterogeneous Acid Catalyst and Its Comparison with Homogeneous Acid and Enzyme Catalysts. *Fuel* **2017**, *187*, 180–188. [CrossRef]
171. Environ, E.; Gu, H.; Jiang, Y.; Zhou, L.; Gao, J. Energy & Environmental Science Reactive Extraction and in Situ Self-Catalyzed Methanolysis of Germinated Oilseed for Biodiesel Production. *Energy Environ. Sci.* **2011**, *4*, 1337–1344. [CrossRef]
172. Prommuak, C.; Pavasant, P.; Quitain, A.T.; Goto, M. Microalgal Lipid Extraction and Evaluation of Single-Step Biodiesel Production. *Eng. J.* **2012**, *16*, 157–166. [CrossRef]
173. Abedini Najafabadi, H.; Vossoughi, M.; Pazuki, G. The Role of Co-Solvents in Improving the Direct Transesterification of Wet Microalgal Biomass under Supercritical Condition. *Bioresour. Technol.* **2015**, *193*, 90–96. [CrossRef] [PubMed]
174. Park, J.; Kim, B.; Lee, J.W. In-Situ Transesterification of Wet Spent Coffee Grounds for Sustainable Biodiesel Production. *Bioresour. Technol.* **2016**, *221*, 55–60. [CrossRef] [PubMed]
175. Ullah, Z.; Sada, A.; Muhammad, N.; Ullah, R.; Alqahtani, A.S.; Nasir, S.; Ben, O.; Azmi, M.; Man, Z. A Review on Ionic Liquids as Perspective Catalysts in Transesteri Fi Cation of Different Feedstock Oil into Biodiesel. *J. Mol. Liq.* **2018**, *266*, 673–686. [CrossRef]
176. Lee, Y.R.; Row, K.H. Comparison of Ionic Liquids and Deep Eutectic Solvents as Additives for the Ultrasonic Extraction of Astaxanthin from Marine Plants. *J. Ind. Eng. Chem.* **2016**, *39*, 87–92. [CrossRef]
177. Suarez Garcia, E.; Suarez Ruiz, C.A.; Tilaye, T.; Eppink, M.H.M.; Wijffels, R.H.; van den Berg, C. Fractionation of Proteins and Carbohydrates from Crude Microalgae Extracts Using an Ionic Liquid Based-Aqueous Two Phase System. *Sep. Purif. Technol.* **2018**, *204*, 56–65. [CrossRef]
178. Young, G.; Nippen, F.; Titterbrandt, S.; Cooney, M.J.; Young, G.; Nippen, F.; Titterbrandt, S.; Cooney, M.J. Direct Transesterification of Biomass Using an Ionic Liquid Co-Solvent System Direct Transesterification of Biomass Using an Ionic Liquid Co-Solvent System. *Biofuels* **2011**, *2*, 261–266. [CrossRef]
179. Orr, V.C.A.; Plechkova, N.V.; Seddon, K.R.; Rehmann, L. Disruption and Wet Extraction of the Microalgae Chlorella Vulgaris Using Room-Temperature Ionic Liquids. *ACS Sustain. Chem. Eng.* **2016**, *4*, 591–600. [CrossRef]
180. Lee, H.; Jung, W.S.J.; Woong, C.; Jae, K.; Kwon, W.L.J. Optimization of Variables Affecting the Direct Transesterification of Wet Biomass from Nannochloropsis Oceanica Using Ionic Liquid as a Co-Solvent. *Bioprocess Biosyst. Eng.* **2015**, *38*, 981–987. [CrossRef] [PubMed]
181. Lai, J.-Q.; Hu, Z.-L.; Wang, P.-W.; Yang, Z. Enzymatic Production of Microalgal Biodiesel in Ionic Liquid [BMIm][PF6]. *Fuel* **2012**, *95*, 329–333. [CrossRef]
182. Durand, E.; Lecomte, J.; Villeneuve, P. Deep Eutectic Solvents: Synthesis, Application, and Focus on Lipase-Catalyzed Reactions. *Eur. J. Lipid Sci. Technol.* **2013**, *115*, 379–385. [CrossRef]
183. Shishov, A.; Bulatov, A.; Locatelli, M.; Carradori, S.; Andruch, V. Application of Deep Eutectic Solvents in Analytical Chemistry. A Review. *Microchem. J.* **2017**, *135*, 33–38. [CrossRef]
184. Pan, Y.; Alam, M.A.; Wang, Z.; Wu, J.; Zhang, Y.; Yuan, Z. Enhanced Esterification of Oleic Acid and Methanol by Deep Eutectic Solvent Assisted Amberlyst Heterogeneous Catalyst. *Bioresour. Technol.* **2016**, *220*, 543–548. [CrossRef]
185. Radošević, K.; Cvjetko Bubalo, M.; Gaurina Srček, V.; Grgas, D.; Landeka Dragičević, T.; Radojčić Redovniković, I. Evaluation of Toxicity and Biodegradability of Choline Chloride Based Deep Eutectic Solvents. *Ecotoxicol. Environ. Saf.* **2015**, *112*, 46–53. [CrossRef]
186. Troter, D.Z.; Todorović, Z.B.; Đokić-Stojanović, D.R.; Stamenković, O.S.; Veljković, V.B. Application of Ionic Liquids and Deep Eutectic Solvents in Biodiesel Production: A Review. *Renew. Sustain. Energy Rev.* **2016**, *61*, 473–500. [CrossRef]
187. Pan, Y.; Alam, M.A.; Wang, Z.; Huang, D.; Hu, K.; Chen, H.; Yuan, Z. One-Step Production of Biodiesel from Wet and Unbroken Microalgae Biomass Using Deep Eutectic Solvent. *Bioresour. Technol.* **2017**, *238*, 157–163. [CrossRef]
188. Vafaeezadeh, M.; Hashemi, M.M. Polyethylene Glycol (PEG) as a Green Solvent for Carbon–Carbon Bond Formation Reactions. *J. Mol. Liq.* **2015**, *207*, 73–79. [CrossRef]

189. Liu, J.; Cao, X.; Chu, Y.; Zhao, Y.; Wu, P.; Xue, S. Novel Approach for the Direct Transesterification of Fresh Microalgal Cells via Micro-Reactor. *Algal Res.* **2018**, *32*, 38–43. [CrossRef]
190. Cui, Y.; Liang, Y. Direct Transesterification of Wet Cryptococcus Curvatus Cells to Biodiesel through Use of Microwave Irradiation. *Appl. Energy* **2014**, *119*, 438–444. [CrossRef]
191. Patil, P.D.; Gnaneswar, V.; Mannarswamy, A.; Cooke, P.; Munson-mcgee, S.; Nirmalakhandan, N.; Lammers, P.; Deng, S. Bioresource Technology Optimization of Microwave-Assisted Transesterification of Dry Algal Biomass Using Response Surface Methodology. *Bioresour. Technol.* **2011**, *102*, 1399–1405. [CrossRef]
192. Liu, B.; Zhao, Z.K. Biodiesel Production by Direct Methanolysis of Oleaginous Microbial Biomass. *J. Chem. Technol. Biotechnol.* **2007**, *780*, 775–780. [CrossRef]
193. Bundhoo, Z.M.A. Microwave-Assisted Conversion of Biomass and Waste Materials to Biofuels. *Renew. Sustain. Energy Rev.* **2018**, *82*, 1149–1177. [CrossRef]
194. Latorre, M.E.; de Escalada Plá, M.F.; Rojas, A.M.; Gerschenson, L.N. Blanching of Red Beet (*Beta vulgaris* L. *Var. conditiva*) Root. Effect of Hot Water or Microwave Radiation on Cell Wall Characteristics. *LWT Food Sci. Technol.* **2013**, *50*, 193–203. [CrossRef]
195. Cheng, J.; Sun, J.; Huang, Y.; Feng, J.; Zhou, J.; Cen, K. Dynamic Microstructures and Fractal Characterization of Cell Wall Disruption for Microwave Irradiation-Assisted Lipid Extraction from Wet Microalgae. *Bioresour. Technol.* **2013**, *150*, 67–72. [CrossRef]
196. Loong, T.C.; Idris, A. One Step Transesteri Fi Cation of Biodiesel Production Using Simultaneous Cooling and Microwave Heating. *J. Clean. Prod.* **2017**, *146*, 57–62. [CrossRef]
197. Idris, A.; Bukhari, A.; Mohd Yusof, N.; Tan, K. A Simultaneous Cooling and Dielectric Heating: An Advanced Technology to Improve the Yield of Lactides. *Prog. Electromagn. Res. Symp. Proc.* **2012**, *1559–9450*, 141–145.
198. Gude, V.G.; Patil, P.; Martinez-Guerra, E.; Deng, S.; Nirmalakhandan, N. Microwave Energy Potential for Biodiesel Production. *Sustain. Chem. Process.* **2013**, *1*, 5. [CrossRef]
199. Li, J.; Dai, J.; Liu, G.; Zhang, H.; Gao, Z.; Fu, J.; He, Y.; Huang, Y. Biochar from Microwave Pyrolysis of Biomass: A Review. *Biomass Bioenergy* **2016**, *94*, 228–244. [CrossRef]
200. Fattah, I.M.; Noraini, M.Y.; Mofijur, M.; Silitonga, A.S.; Badruddin, I.A.; Khan, T.M.; Mahlia TM, I. Lipid Extraction Maximization and Enzymatic Synthesis of Biodiesel from Microalgae. *Appl. Sci.* **2020**, *10*, 6103. [CrossRef]
201. Ido, A.L.; Daniel, M.; De Luna, G.; Capareda, S.C.; Maglinao, A.L.; Nam, H. Application of Central Composite Design in the Optimization of Lipid Yield from Scenedesmus Obliquus Microalgae by Ultrasound-Assisted Solvent Extraction. *Energy* **2018**, *157*, 949–956. [CrossRef]
202. Mofijur, M.; Kusumo, F.; Fattah, I.M.R.; Mahmudul, H.M.; Rasul, M.G.; Shamsuddin, A.H.; Mahlia, T.M.I. Resource Recovery from Waste Coffee Grounds Using Ultrasonic-Assisted Technology for Bioenergy Production. *Energies* **2020**, *13*, 1770. [CrossRef]
203. Toro, P.H.C.; Navia, G.C.R. Advances in Direct Transesterification of Microalgal Biomass for Biodiesel Production. *Rev. Environ. Sci. Bio/Technol.* **2013**, *12*, 179–199. [CrossRef]
204. Martinez-Guerra, E.; Gude, V.G.; Mondala, A.; Holmes, W.; Hernandez, R. Microwave and Ultrasound Enhanced Extractive-Transesterification of Algal Lipids. *Appl. Energy* **2014**, *129*, 354–363. [CrossRef]
205. Cercado, A.P.; Ballesteros, F.; Capareda, S. Ultrasound Assisted Transesterification of Microalgae Using Synthesized Novel Catalyst. *Sustain. Environ. Res.* **2018**, *28*, 234–239. [CrossRef]
206. Gude, V.G. Synergism of Microwaves and Ultrasound for Advanced Biorefineries. *Resour. Technol.* **2015**, *1*, 116–125. [CrossRef]
207. Ma, G.; Hu, W.; Pei, H.; Jiang, L.; Song, M.; Mu, R. In Situ Heterogeneous Transesterification of Microalgae Using Combined Ultrasound and Microwave Irradiation. *Energy Convers. Manag.* **2015**, *90*, 41–46. [CrossRef]
208. Martinez-Guerra, E.; Howlader, M.S.; Shields-Menard, S.; French, W.T.; Gude, V.G. Optimization of Wet Microalgal FAME Production from Nannochloropsis Sp. under the Synergistic Microwave and Ultrasound Effect. *Int. J. Energy Res.* **2018**, *42*, 1934–1949. [CrossRef]
209. Duarte, S.H.; dos Santos, P.; Michelon, M.; de Pinho Oliveira, S.M.; Martínez, J.; Maugeri, F. Recovery of Yeast Lipids Using Different Cell Disruption Techniques and Supercritical CO_2 Extraction. *Biochem. Eng. J.* **2017**, *125*, 230–237. [CrossRef]
210. Jafari, A.; Esmaeilzadeh, F.; Mowla, D.; Sadatshojaei, E.; Heidari, S.; Wood, D.A. New Insights to Direct Conversion of Wet Microalgae Impregnated with Ethanol to Biodiesel Exploiting Extraction with Supercritical Carbon Dioxide. *Fuel* **2021**, *285*, 119199. [CrossRef]
211. Kim, J.Y.; Jung, J.-M.; Jung, S.; Park, Y.-K.; Tsang, Y.F.; Lin, K.-Y.A.; Choi, Y.-E.; Kwon, E.E. Biodiesel from Microalgae: Recent Progress and Key Challenges. *Prog. Energy Combust. Sci.* **2022**, *93*, 101020. [CrossRef]
212. Goto, M.; Kanda, H.; Wahyudiono; Machmudah, S. Extraction of Carotenoids and Lipids from Algae by Supercritical CO_2 and Subcritical Dimethyl Ether. *J. Supercrit. Fluids* **2015**, *96*, 245–251. [CrossRef]
213. Tobar, M.; Núñez, G.A. The Journal of Supercritical Fluids Supercritical Transesteri Fi Cation of Microalgae Triglycerides for Biodiesel Production: E Ff Ect of Alcohol Type and Co-Solvent. *J. Supercrit. Fluids* **2018**, *137*, 50–56. [CrossRef]
214. Reddy, H.K.; Muppaneni, T.; Patil, P.D.; Ponnusamy, S.; Cooke, P.; Schaub, T.; Deng, S. Direct Conversion of Wet Algae to Crude Biodiesel under Supercritical Ethanol Conditions. *Fuel* **2014**, *115*, 720–726. [CrossRef]
215. Mohamadzadeh, H.; Karimi-sabet, J.; Ghotbi, C. Bioresource Technology Biodiesel Production from Spirulina Microalgae Feedstock Using Direct Transesterification near Supercritical Methanol Condition. *Bioresour. Technol.* **2017**, *239*, 378–386. [CrossRef]

216. Patil, P.D.; Gnaneswar, V.; Mannarswamy, A.; Cooke, P.; Nirmalakhandan, N.; Lammers, P.; Deng, S. Comparison of Direct Transesterification of Algal Biomass under Supercritical Methanol and Microwave Irradiation Conditions. *Fuel* **2012**, *97*, 822–831. [CrossRef]
217. Crampon, C.; Nikitine, C.; Zaier, M.; Lépine, O.; Tanzi, C.D.; Vian, M.A.; Chemat, F.; Badens, E. Oil Extraction from Enriched Spirulina Platensis Microalgae Using Supercritical Carbon Dioxide. *J. Supercrit. Fluids* **2017**, *119*, 289–296. [CrossRef]
218. Li, J.; Si, B.; Liao, Q.; Fu, Q.; Liu, Z. Insights into Hydrothermal Process of Microalgae via Novel Modified Kinetic Model and Thermodynamic Analysis. *Fuel* **2022**, *317*, 123540. [CrossRef]
219. Chiaramonti, D.; Prussi, M.; Buffi, M.; Maria, A.; Pari, L. Review and Experimental Study on Pyrolysis and Hydrothermal Liquefaction of Microalgae for Biofuel Production. *Appl. Energy* **2017**, *185*, 963–972. [CrossRef]
220. Kim, B.; Park, J.; Son, J.; Lee, J.W. Bioresource Technology Catalyst-Free Production of Alkyl Esters from Microalgae via Combined Wet in Situ Transesteri Fi Cation and Hydrothermal Liquefaction (ITHL). *Bioresour. Technol.* **2017**, *244*, 423–432. [CrossRef] [PubMed]
221. Marangon, B.B.; Castro, J.S.; Assemany, P.P.; Couto, E.A.; Calijuri, M.L. Environmental Performance of Microalgae Hydrothermal Liquefaction: Life Cycle Assessment and Improvement Insights for a Sustainable Renewable Diesel. *Renew. Sustain. Energy Rev.* **2022**, *155*, 111910. [CrossRef]
222. Masoumi, S.; Dalai, A.K. Techno-Economic and Life Cycle Analysis of Biofuel Production via Hydrothermal Liquefaction of Microalgae in a Methanol-Water System and Catalytic Hydrotreatment Using Hydrochar as a Catalyst Support. *Biomass Bioenergy* **2021**, *151*, 106168. [CrossRef]
223. Chen, P.H.; Quinn, J.C. Microalgae to Biofuels through Hydrothermal Liquefaction: Open-Source Techno-Economic Analysis and Life Cycle Assessment. *Appl. Energy* **2021**, *289*, 116613. [CrossRef]
224. Zhou, Y.; Schideman, L.; Yu, G.; Zhang, Y. A Synergistic Combination of Algal Wastewater Treatment and Hydrothermal Biofuel Production Maximized by Nutrient and Carbon Recycling. *Energy Environ. Sci.* **2013**, *6*, 3765–3779. [CrossRef]
225. Kumar, L.; Anand, R.; Shah, M.P.; Bharadvaja, N. Microalgae Biodiesel: A Sustainable Source of Energy, Unit Operations, Technological Challenges, and Solutions. *J. Hazard. Mater. Adv.* **2022**, *8*, 100145. [CrossRef]
226. Kim, T.; Oh, Y.; Lee, J.W.; Keun, Y. Levulinate Production from Algal Cell Hydrolysis Using in Situ Transesteri Fi Cation. *Algal Res.* **2017**, *26*, 431–435. [CrossRef]
227. White, R.; Ryan, R. Long-Term Cultivation of Algae in Open-Raceway Ponds: Lessons from the Field. *Ind. Biotechnol.* **2015**, *11*, 213–220. [CrossRef]
228. Nishshanka, G.K.S.H.; Anthonio, R.A.D.P.; Nimarshana, P.H.V.; Ariyadasa, T.U.; Chang, J.-S. Marine Microalgae as Sustainable Feedstock for Multi-Product Biorefineries. *Biochem. Eng. J.* **2022**, *187*, 108593. [CrossRef]
229. Lim, J.Y.; Teng, S.Y.; How, B.S.; Nam, K.; Heo, S.; Máša, V.; Stehlík, P.; Yoo, C.K. From Microalgae to Bioenergy: Identifying Optimally Integrated Biorefinery Pathways and Harvest Scheduling under Uncertainties in Predicted Climate. *Renew. Sustain. Energy Rev.* **2022**, *168*, 112865. [CrossRef]
230. Sheehan, J.; Dunahay, T.; Benemann, J.; Roessler, P. A Look Back at the US Department of Energy's Aquatic Species Program: Biodiesel from Algae. *Natl. Renew. Energy Lab.* **1998**, *328*, 1–294.
231. Mourya, M.; Khan, M.J.; Ahirwar, A.; Schoefs, B.; Marchand, J.; Rai, A.; Varjani, S.; Rajendran, K.; Banu, J.R.; Vinayak, V. Latest Trends and Developments in Microalgae as Potential Source for Biofuels: The Case of Diatoms. *Fuel* **2022**, *314*, 122738. [CrossRef]
232. Martín, L.A.; Popovich, C.A.; Martínez, A.M.; Scodelaro, P.G.; Damiani, M.C.; Leonardi, P.I. Hybrid Two-Stage Culture of Halamphora Coffeaeformis for Biodiesel Production: Growth Phases, Nutritional Stages and Biore Fi Nery Approach. *Renew. Energy* **2018**, *118*, 984–992. [CrossRef]