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Review

Sustainable Approaches to Microalgal Pre-Treatment Techniques for Biodiesel Production: A Review

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Abstract: Microalgae are a potential source of numerous nutritional products and biofuels. Their applications range from the food industry to the medical and fuel sectors and beyond. Recently, the conversion of biomass into biodiesel and other biofuels has received a lot of positive attention within the fossil fuel arena. The objective of biorefineries is to focus on utilising biomass efficiently to produce quality biofuel products by minimising the input as well as to reduce the use of chemical or thermal pre-treatments. Pre-treatment processes in biorefineries involve cell disruption to obtain lipids. Cell disruption is a crucial part of bioconversion, as the structure and nature of microalgae cell walls are complex. In recent years, many research papers have shown various pre-treatment methods and their advantages. The objective of this paper was to provide a comprehensive in-depth review of various recent pre-treatment techniques that have been used for microalgal biodiesel production and to discuss their advantages, disadvantages, and how they are applied in algal biorefineries.

Keywords: biofuel; microalgae; pre-treatment; biomass; cell disruption; lipids; biodiesel; renewable energy

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1. Introduction

Natural resources are a significant part of the economic structures that meet the requirements of humanity. With the increasing human population, economic production is also constantly growing, which paves the way for research into the creation of new products and the innovation of current materials in an attempt to overcome the energy crisis. The energy crisis is one of the greatest current concerns for the world's stability and peace. Countries with developing economies that have limited natural resources need to secure fuel supplies. Fossil fuels, such as coal, petrol, natural gas, etc., have been viewed as fundamental energy sources [1], and they are used in very large amounts around the world. However, our long-term dependence on fossil fuels has challenged the lowering of greenhouse gases and has paved the way for global warming. The increase in the earth's overall temperature due to various human activities and natural causes has also contributed to the phenomenon of global warming. Some data have shown that the increase in the global temperature may result in increased health risks in future generations [2]. In order to retain clean ecosystems and maintain stability, renewable and eco-friendly biofuels are needed to replace fossil fuels [3]. These replacements are derived from natural resources, such as microalgae [4]. Algae comprise macroscopic and microscopic organisms, with some macroscopic organisms growing to a length of 10 m, and some microscopic organisms growing to a few micrometres in size.

Microalgae are considered to be a fascinating resource for industries, as they are helpful for producing multitudinous products because of their high growth rate, photosynthesis efficiency, and process optimisation. They have already been used in commercial industries, such as in animal feed, food, therapeutics, cosmetics, and biofuel [5–9]. The main advantages of culturing microalgae are that they can be cultured with minimal

space, fewer nutrients, and minimal water (saline or brackish water) [10,11]. Microalgal cells are relatively small and are protected inside the cell wall. The Golgi apparatus within the cells contain certain products, and in some species, these products are to bound to the cell membranes. Due to these complex cell wall structures, cell disruption can be challenging [12,13]. Some microalgae species are easy to break down using a mild and effective cell disruption techniques, but this may not support large-scale production. Therefore, it is important to compare and choose feasible and energy-efficient cell disruption methods to obtain the highest standard of the extracted products, the most economical operating costs, and the highest lipid recovery rates.

In recent years, microalgae have gained more attention than most other sources of extractable biofuel. Biofuels are defined as fuels that are produced from agricultural or forestry materials or from the biodegradable parts of industrial waste [14]. Biofuel extraction has been calculated as producing 35 billion litres of fuel [15], with the USA, Brazil, and the European Union being the top producers [16]. Biodiesel is produced from vegetable oils [17], jatropha curcas [18], biobutanol [19], and algae [20]. Biodiesel can be generated from microalgal cell disruption using pre-treatment techniques that help to extract lipids. It is also clear that microalgae can produce large amounts of lipids. Table 1 shows some examples of algae species and the lipids extracted from them following pre-treatment.

The biological value of microalgal oil and biomass is due to their ability to synthesise a variety of elements, their growth capacity, and their capability to increase the efficiency of targeted bio-compounds using cultivation parameters and extraction methodologies [21,22]. Today, there is an increasing demand for algal biomass due to the increase in both traditional industries and microalgal applications. Estimates have shown that *Chlorella* and *Arthrospira* production has reached 2000–5000 tons and 6700–12,000 tons, respectively [23,24]. The higher initial costs of mass microalgal cultivation and the associated raw materials pose a problem for large-scale biomass production; however, reducing these costs and enhancing the economic productivity of microalgal lipids can be achieved using different techniques. The use of eco-friendly pre-treatment techniques such as mechanical [25], microwave [26], chemical [27,28], ultrasonic [29], high-pressure homogenisation [30] has been extensively studied. During cultivation, the use of genetic engineering processes to increase the recovery of lipids, proteins, and other valuable bioproducts is considered to be quite challenging [31]. This review paper focused on an analysis of research studies on the standard pre-treatment methods that are already in use as well as emerging techniques. All of the existing lipid extraction methods were analysed and compared using different species of microalgae.

2. Pre-Treatments

In various research studies from around the globe, techniques for pre-treating microalgae are still in development while researchers try to acquire more efficient lipid products (Table 1). The cell disruption technique involves breaking down the cells within the cell membranes to remove the intercellular products. It has also been shown that pre-treatment processes consume a lot of energy during cell disruption [32]. Some microalgal species are naturally good for efficient lipid extraction, but that does not apply to all types of algae. In order to produce significant cell disruption, the right pre-treatment method must be chosen to enhance the disruption efficiency [33]. Recent studies have shown that many new cell disruption techniques and methods are involved in the development of bioethanol and biofuels. The major obstacle to the use of biofuels has been the operational costs of large-scale production because the production of biofuels requires more input products (Figure 1) [34]. However, biofuels are emerging as non-toxic alternative fuel resources that are less harmful to the environment [20]. The use of biodiesel is increasing gradually, and as the demand rises, more pre-treatment methods and techniques are required [35].

Table 1. Lipids extracted after pre-treatment in microalgae.

Microalgae	Lipid Productivity (mg/L/day)	References
Phaeodactylum tricomutum	44.8	[36]
Chaetoceros muelleri	21.8	[37]
Skeletonema costatum	17.4	[37]
Botryococcus braunii	5.5	[38]
Dunaliella tertiolecta	60.6–69.8	[37]
Dunaliella sp.	33.5	[36]
Dunaliella salina	116	[36]
Nannochloris sp.	60.9–76.5	[37]
Nannochloropsis sp.	54.8	[38]
Nannochloropsis oculata	84.0–142.0	[36]
Scenedesmus sp.	40.8–53.9	[36]
Chlorella sp.	42.1	[38]
Chlorella vulgaris	11.2–40.0	[36]
Chlorella protothecoides	1214	[36]
Chlorella emersonii	10.3–50.0	[36]
Pavlova salina	49.4	[36]

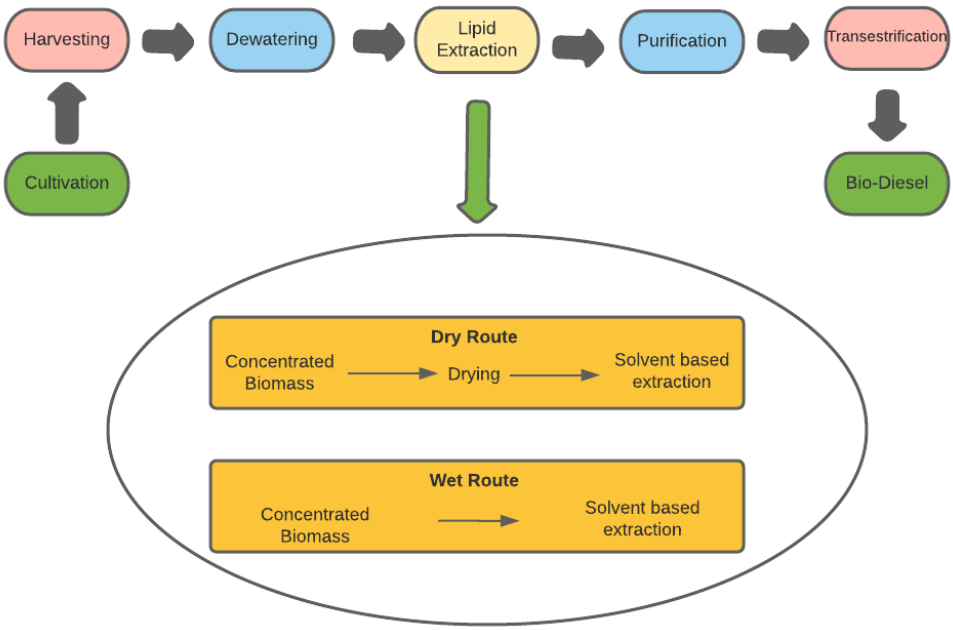

Figure 1. Approaches for converting microalgae to biodiesel.

A variety of pre-treatment techniques are currently used for cell disruption. These methods are mainly classified into mechanical, physical, thermal, chemical, biological, pulsed electric field, and combined techniques. Mechanical processes are the most widely used methods to reduce the rate of shear force required for cell wall rupture. In recent years, microwave, catalytic, bead beating, autoclaving, enzymatic, ultrasonic, autoclave, steam explosion, high-pressure homogenisation, high-speed homogenisation, and sonication methods have been studied for use in biodiesel applications and have shown good economic efficiency outcomes in large-scale production. It should be noted that the same microalgae can produce divergent lipid productivity results when using different pre-treatment techniques. As such, it is better to conduct a systematic evaluation on how the pre-treatment method influences the cell wall and cell size of a specific microalgae before

choosing a certain technique [39]. When comparing the production processes of pre-treated biomass with non-pre-treated biomass, the energy balance favours the former [40].

2.1. Mechanical Pre-Treatment

Mechanical pre-treatment techniques involve the destruction of the cell wall using shear forces. Mechanical pre-treatment techniques can be classified into the categories of high-pressure homogenisation, high-speed homogenisation, and bead milling [41–43]. These methods are proven to extract lipids in a way that enhances large-scale biofuel production [44,45]. The main objective of these methods is to reduce cell wall particle size and crystallinity at the time of cell disintegration [46]. Mechanical pre-treatment methods have the advantage of preventing the cells from being contaminated and protect cell function from being damaged during cell rupture [47]. These methods become more effective when used in a combination, and they increase the cell surface area and produce more disruption efficiency [48].

2.1.1. High-Pressure Homogenisers

High-pressure homogenisers (HPHs) are specially made for emulsification techniques. This method is broadly used for the microalgae cell disruption process because of its continuous operation and scalability to generate wet biomass [49]. The HPH method has been shown to recover more microalgae lipids during cell rupture [50]. Different types of valve seat formats are available to optimise cell disruption efficiency [51]. The cells flow through the valve and strike the impact ring, exit the valve, and are discharged. Here, cell disruption is achieved through shear forces due to the impact caused, and hydrodynamic cavitation shows a pressure drop (Figure 2) [52]. HPHs show a higher possibility of obtaining wet microalgae concentrates with 25 W/W% solids for lipid recovery efficiently without consuming more energy [53]. In industries, pre-treatment methods using high-pressure homogenisers are used for cell disruption in seaweeds and yeast cells to improve lipid production [54]. An overview of research studies using this method is shown and discussed in Table 2.

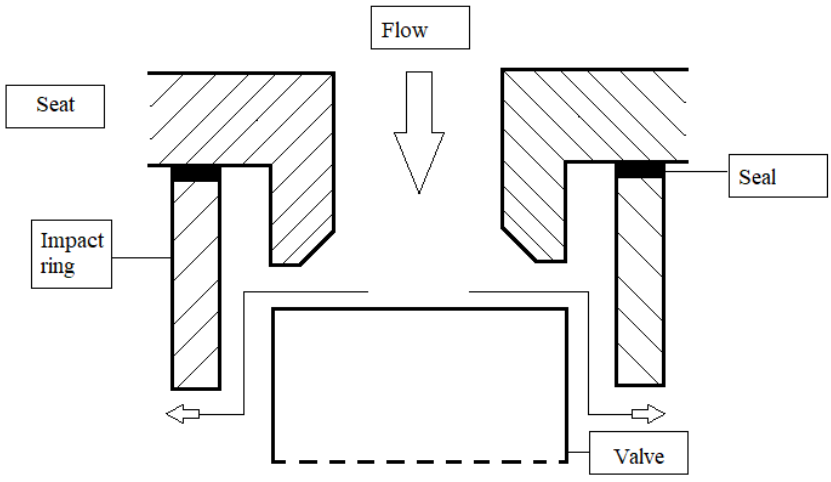

Figure 2. Schematic diagram of HPH value seat.

Table 2. Overview of previous research studies using high-pressure homogenisers.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Nannochloris oculata	68.9 MPa and 310 MPa using nozzle diameters of 130 mm and 185 mm, respectively, 6 passes–100 mL	Efficiency increased.	Biodiesel production, total lipids.	[53]
	1%DCW, 125 MPa, 5 passes.	Efficiency of 200 (mg/g cell)	Biodiesel production, total lipids.	[55]
Chlorococcum sp.	0.85% DCW, 8 Mpa, 4 passes–200 mL	90% cell disruption achieved	Perfect cell count, total lipids.	[52]
Tetraselmissuecica	86 MPa. 0.85% DCW, 5 passes–200 mL	34.157 cell/mm^3 cell concentration	Perfect cell count, total lipids.	[52]
Auxenochlorella protothecoides	150 Mpa, 5 passes. Energy input of 1.5 MJ/kg dry weight–40 mL	Yields up to 35% (dry weight)	Perfect cell count, protein analysis	[56]
Chlorella vulgaris	150 Mpa, 5 passes. Energy input of 1.5 MJ/kg dry weight–40 mL	~25% (dry weight) protein release	Perfect cell count, protein analysis	[56]
Nannochloropsis sp.	150 Mpa, 1% DCW, nitrogen added, 6 passes–250 mL	90% protein achieved	Protein analysis	[57]
Chlorella saccharophila	200 to 1000 bar, t-butanol, ammonium sulphate.	Efficiency of 400 (mg/g cell)	Perfect cell count, total lipids.	[58]

The list of studies in the table shows that increasing the pressure and number of cycles will have a good impact on the lipid efficiency. Some studies suggest that lowering the dry cell weight and culture stress levels seems important but that modifying the nozzle diameter does not seem very effective [52]. Even though the use of HPHs is the most preferred method, they do have some disadvantages as well. When using a low DCW (0.01–0.85% *w*/*w*), the energy demand increases, and the hard cells become challenging to break. This indicates that HPH methods are not mild methods and are not acceptable for breaking fragile elements [59].

2.1.2. Bead Milling

Bead mills are widely used for lipid extraction during microalgae cell disruption. They provide good disruption efficiency in a single pass, and their industrial implementation values include temperature maintenance, easy operating procedures, large biomass set up, and easily available equipment [60]. Figure 3 shows the basic components of bead mills. They are classified into two types: agitated vessels and shaking vessels. Shaking vessels can be used to disrupt cells by vibrating the whole vessel. Agitated vessels use a spinning agitator that is filled with cell culture and beads. The cell disruption rate depends on size of the beads, the rigidity of the cell, and the biomass material of the microalgal cell [25]. It is theorised that after shear force is applied, the cells will be disrupted in the bead collision zones, and the energy will be transferred from the beads to the cells [61,62]. An overview of the literature is shown in Table 3 and discussed below.

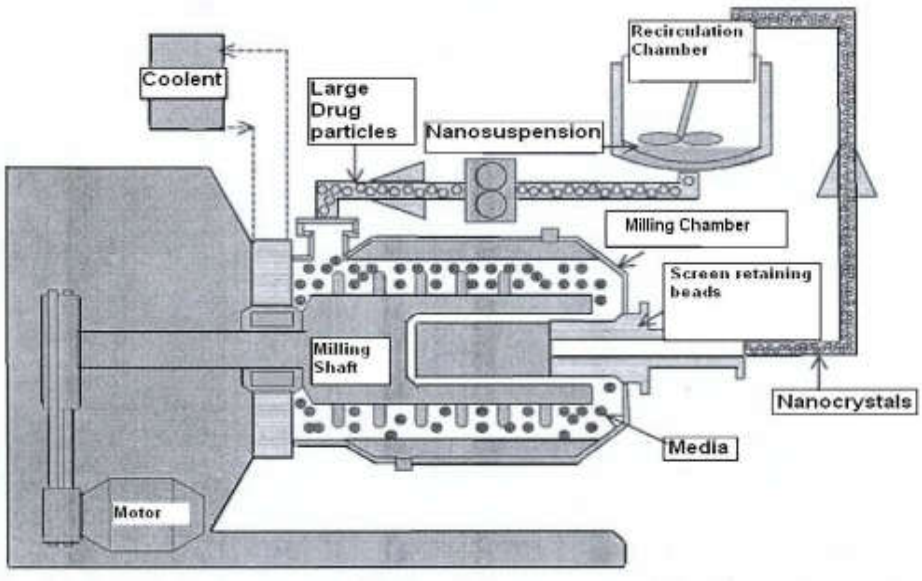

Figure 3. Schematic view of a wet bead milling process [63].

Table 3. Overview of the previous research studies using bead milling.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Chlorella vulgaris	25 gDW L^{-1} biomass concentration, 2039 rpm, protease and cellulase (2% *v/w*, 1:1), 45 °C, 24 h–75 mL	75% lipid recovery (solid phase)	Perfect cell count, total lipids.	[64]
Nannochloropsis sp.	3 kw 0.5 beads, 4500 rpm/10 min–25 mL	Highest biomass concentration and COD reduction of 1.268 g/L and 71%, respectively	Perfect cell count, total lipids.	[65]
Chlorella vulgaris	Speed of the agitator set at 10 m^{-1} and a power of 24.5 kW for 90 min	95% increase in cell disruption	Perfect cell count, total lipids.	[66]
	3.3 kW, 0.40–0.50 mm beads, 10.7% dry cell weight–1.4 L	99% cell disintegration	Perfect cell count, total lipids	[67]
	(25–145 gDW kg^{-1}) and agitator speeds (6–12 m s^{-1})	97% cell disintegration	Perfect cell count, protein analysis	[68]
Nannochloropsis oculata	175 MPa, chloroform, methanol	Efficiency of 2.8 (mg/g cell)	Perfect cell count, total lipids.	[69]

Based on the case studies from the literature, various factors such as feed rate suspension, continuous operation, bead diameter, bead density, milling chamber design, biomass concentration, agitator design, agitator speed, bead filling, and the processing time of each batch affects the cell disruption rate during bead mill pre-treatment processes [70]. It can be also said that increasing the size of the beads will show more effective results than using small (0.5 mm) beads. The selected case studies also show that it is best to use low-density beads for low-viscosity media and high-density beads for high-viscosity media [42,67]. In spite of their advantages, their cons include their high energy demands and high operational costs, which makes this method less preferred for industries.

2.1.3. High-Speed Homogenisers

High-speed homogenisers (HSH) are devices that use a stirring mechanism that rotates at very high rpm and that consist of rotors and stators that are preferably assembled out of stainless steel. Cell disruption occurs when the cutting spindle rotates at a high

speed, causing hydrodynamic cavitation and high shear forces inside microalgal cells by breaking their cell walls and extracting the intercellular elements from them. According to cell wall characteristics, operating conditions such as the homogenising speed, number of passes, and running period can be optimised to increase efficiency [71]. Additionally, other factors such as the microalgal species, dry cell concentration, and growth parameters influence the energy consumption and the efficiency of the pre-treatment process. A reduction in the biomass size due to the high pressure in the homogeniser causing a thermal effect on the sample results in the aggregation of the biocompounds and their release into to the aqueous media used as references [72–74]. The HSH technique is a very simple but very aggressive cell disruption technique that achieves effective results. The main advantage of this process is its short operating time and its potential to generate lipids and other compounds. Some of the research was conducted to increase the extraction yield using different species and biochemicals [75]. The main disadvantages of this technique are the high operational costs, the protein denaturation caused due to the shear force, and the increase thermal effect, and these make this technique less favourable for biorefinery industries [41]. An overview of studies from the literature using this method is shown in Table 4.

Table 4. Overview of previous research studies using HSH.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Porphyridium cruentum	5500 rpm for 10 min	ω3-PUFA food products	Perfect cell count.	[72]
Synechococcus sp.	10,000 rpm for 1 min (5 cycles)	8.82% lipid recovery	Total lipids.	[74]
Laminaria digitata	150–500 bar for 15 min	20% lipid content	Perfect cell count, total lipids.	[76]
Chlorella sp.	12,000 rpm for 15 min	Lipid efficiency of 13.05%	Perfect cell count, total lipids.	[71]
Nannochloropsis sp.	Speed of 10,000 rpm–1 min–15 mL	Dry extraction yield of 75%	Total lipids.	[77]

2.2. Physical Pre-Treatment

Physical pre-treatments involve the application of mechanical forces such as shear force, microwaves, and ultrasound. Physical pre-treatment methods have advantages such as cost effectiveness, ease of commercialisation, and time saving. They consist of two major classifications: microwave and ultrasound methods.

2.2.1. Ultrasound Pre-treatment

During ultrasonic pre-treatment, acoustic or sound energies of high-frequency waves are generated. By transmitting these shock waves into the cell wall, they cause cell disruption because of their high shear force. The pressure variation in these waves can produce cavitation within the cell [78,79]. The impact of ultrasound waves is mostly influenced by the cell wall structure and composition of the microalgae (Figure 4). Because of high temperature and pressure levels, the cavitation generates chemical reactions that are able to destroy organic matter and produce shear force, leading to the creation of H^+ and OH^- reactive radicals [80].

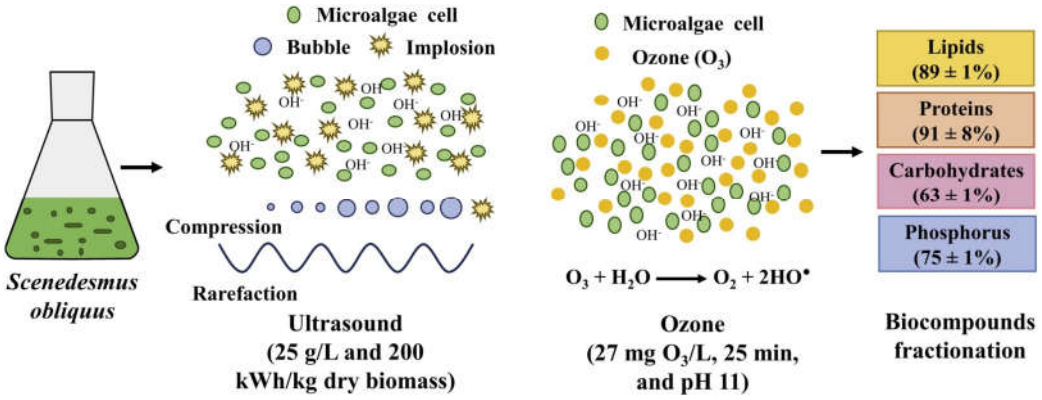

Figure 4. Graphic showing *Scenedesmus obliquus* during ultrasonic pre-treatment [81].

The ultrasound method is used in various applications, such as in olive mill wastewater, chicken and cattle manure, and sludge [82,83]. Microalgae biomass efficiency has been successfully increased to between 16% and 100% with high acoustic energy input. One study observed that there was no improvement in *spirulina maxima* when a semicontinuous reactor was used,. This was mainly because of the characteristics of microalgae, which have a soft cell wall [84]. High temperatures should be avoided when using the ultrasound technique, as they result in the loss of volatile organics and reduce biomass production. This was suggested during a study with *Nannochloropsis salina,* which showed lower biomass yield when compared to raw biomass [85]. The schematic representation of the ultrasonic machine is shown in Figure 5. A review of case studies using this method is shown in Table 5.

Table 5. Overview of the previous research studies using ultrasonic methods.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Botryococcus sp.	0.5%DCW, 5 min, 10 kHz–100 mL	8.8% lipid recovery	Total lipids.	[54]
Salvinia molesta.	5 min and frequency of 2 kHz–100 mL	19.7% increased lipid content	Perfect cell count, total lipids.	[86]
Chlorella vulgaris	20 kHz using 750 W for different times: 0, 5, 10, and 20 min, at 25 °C	23% lipid content	Perfect cell count, total lipids.	[63]
Chlorella sp.	20 kHz, 0.8 kW-h/L cells	Efficiency of 12.6 (mg/g cell)	Perfect cell count, total lipids.	[87]
Schizochytrium sp.	150 W, time for 30 min, with temperature 50 °C	Oil yields up to 93.76 (dry weight)	Total lipids.	[88]
Scenedesmus sp.	20 W and frequency 18 Hz for 5 s.	21.3% to 28.3% lipid yield	Perfect cell count, protein analysis	[64]

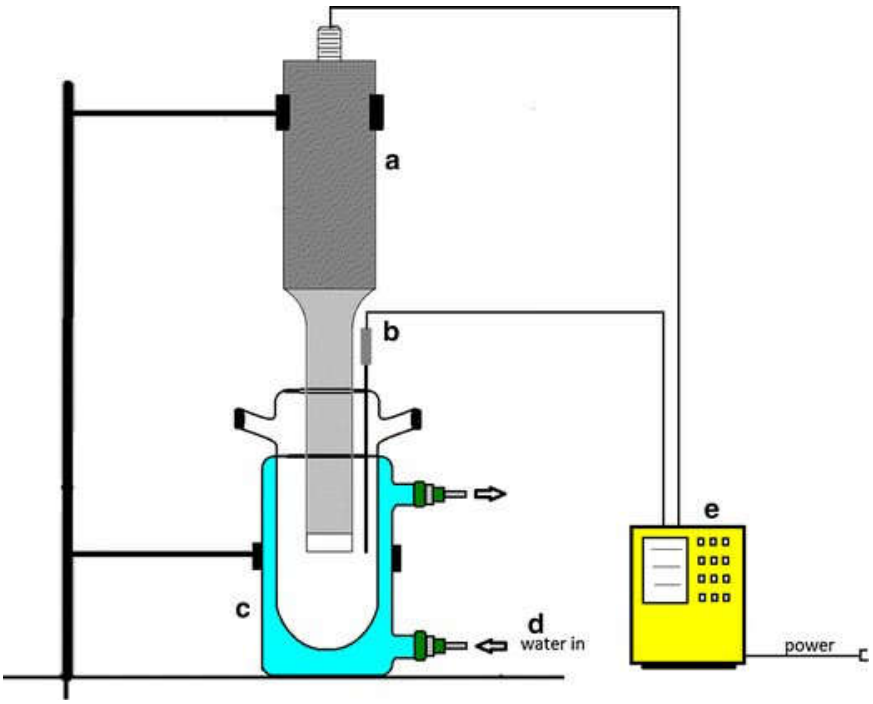

Figure 5. Schematic representation of ultrasonic system: (**a**) transducer, (**b**) thermometer, (**c**) ultrasonic reactor, (**d**) cryostat, (**e**) ultrasonic generator [89].

When pre-treating with ultrasonication, the working temperature significantly increases from around 50 to 90 °C, which also kills proteins and other intercellular elements [90]. The disadvantage of this method is its low disruption efficiency. Lipid quality can be increased with temperature control, but this decreases the efficiency slightly [66].

2.2.2. Microwave Techniques

Microwave techniques use optimal electromagnetic waves with frequencies ranging from 0.3 to 300 GHz that are used to heat localised areas. Here, the microwaves increase the kinetic energy of water molecules until they reach their boiling state [91]. Microwave treatments produce thermal radiation, and this effect is said to increase the temperature due to polarised macro molecules (Figure 6). These modules are aligned around the pole of the electromagnetic field, which is where hydrogen ions break down [26]. The pressure and the heat energy produced by microwaves cause damage to the cell wall and cell membranes [92]. A review of case studies using microwave techniques is shown in Table 6.

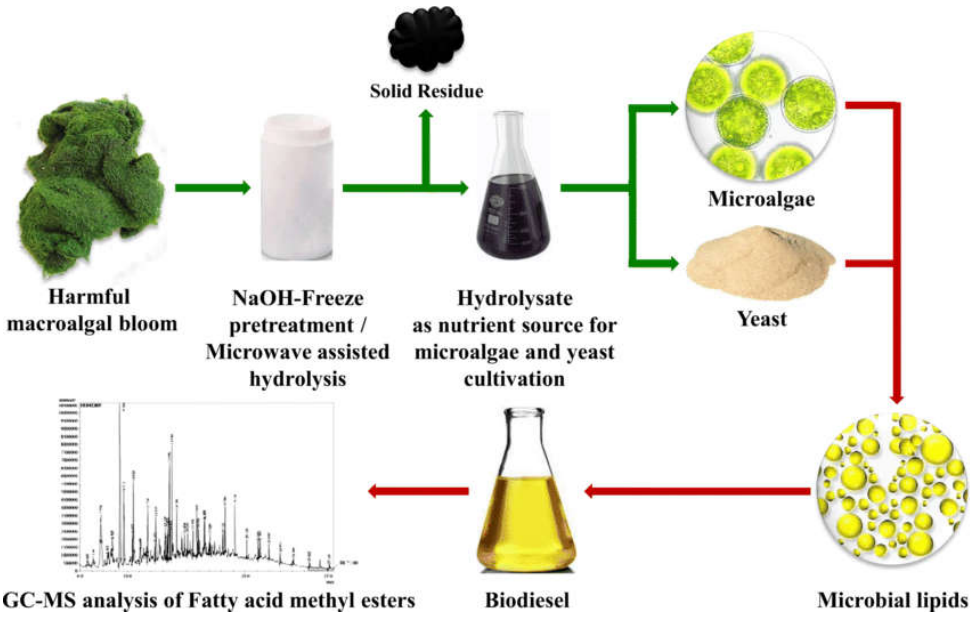

Figure 6. Picture of microwave-assisted treatment for microalgae [93].

Table 6. Overview of the previous research studies using microwave techniques.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Nannochloropsis oceanica	Power of 1025 W and frequency of 245 MHz for 15 min	38.46% lipid production	Total lipids.	[94]
Yarrowia lipolytica	900 W power and a frequency of 245 MHz for 15 min	Lipid production of 8.18%	Total lipids.	[72]
Chlorella sp.	For 15 min, 450 W power. Biomass and methanol ratio of 1:12 (*w*/*v*), catalyst: KOH	32.18% lipid content	Biodiesel, total lipids.	[72]
	Power of 450 W, time of 60 min. Catalyst: 0.2 M H_2SO_4, 5 min	75.68% (FAME for biodiesel production)	Biodiesel, total lipids.	[95]
	2450 MHz and temperature of 100 °C, 5 min	Increased lipid efficiency.	Total lipids.	[96]
Botryococcus braunii	Power 1250 W and frequency 2450 MHz at 150 °C for 20 min	Enchanted lipid efficiency.	Biodiesel production, total lipids.	[73]
Nannochloropsis sp.	65 °C–25 min	42.22% dry biomass yield for biodiesel	Biodiesel production, total lipids.	[97]
	1.2 kW power and frequency of 2.45 GHz. 5–15 min.	Increased cell disruption efficiency.	Total lipids.	[98]
Nannochloropsis oculata	140 °C, 15 min	Lipid content increase: 6.25-fold	Perfect cell count, total lipids.	[99]

From the list of studies, the potential of using more specific energy can be more effective when using microwave techniques. Regardless, cell disruption occurs when water-based substances are observed by microwaves and are formed as head radicals [100]. Compared to normal heating, the microwaves will be uniform during temperature transmission. This method is shown to be more superior compared to the bead milling and ultrasound methods [101]. Even though these techniques have more advantages, they lack

in terms of extraction yield the time required to use solvents. As such, the microwave method is well-suited for use as a mild microalgae cell disruption method [96].

2.3. Thermal Pre-Treatment

Thermal pre-treatment methods are techniques in which heat is added to the surface of algal biomass. This makes the microalgae disrupt the chemical bonds inside their cells, improving the solubilisation [102]. They provide high biomass yields and have low energy requirements when compared to other physical pre-treatment methods. Thermal pre-treatments are basically carried out by adding alkali or acidic chemicals to improve the cell disruption efficiency. Despite these high thermal properties, they may produce recalcitrant components that result in low biomass production and cannot be degraded anaerobically [103]. These methods are categorised into two types: steam explosion and autoclaving.

2.3.1. Steam Explosion

Steam explosion is an economical and effective method that is used in the processing of lignocellulosic components to improve the biomass efficiency. This method uses high temperatures ranging from about 160 °C to 260 °C (1.03–3.40 MPa) [104]. By using a catalyst such as NaOH or H_2SO_4, it is possible to obtain enhanced lipid efficiency [105]. Particle size, chemical composition, and shapes can be modified via explosive depressurisation and autohydrolysis [106,107]. In the search for the best method for cell disruption to enhance efficiency and to extract sugars and carbohydrates, the steam explosion technique is said to be perfect. The application of steam explosion with the acid catalyst method can efficiently extract more lipids [108]. Schematic diagram of steam explosion and a fractionation reactor is shown in Figure 7. A review of the case studies using steam explosion is shown in Table 7.

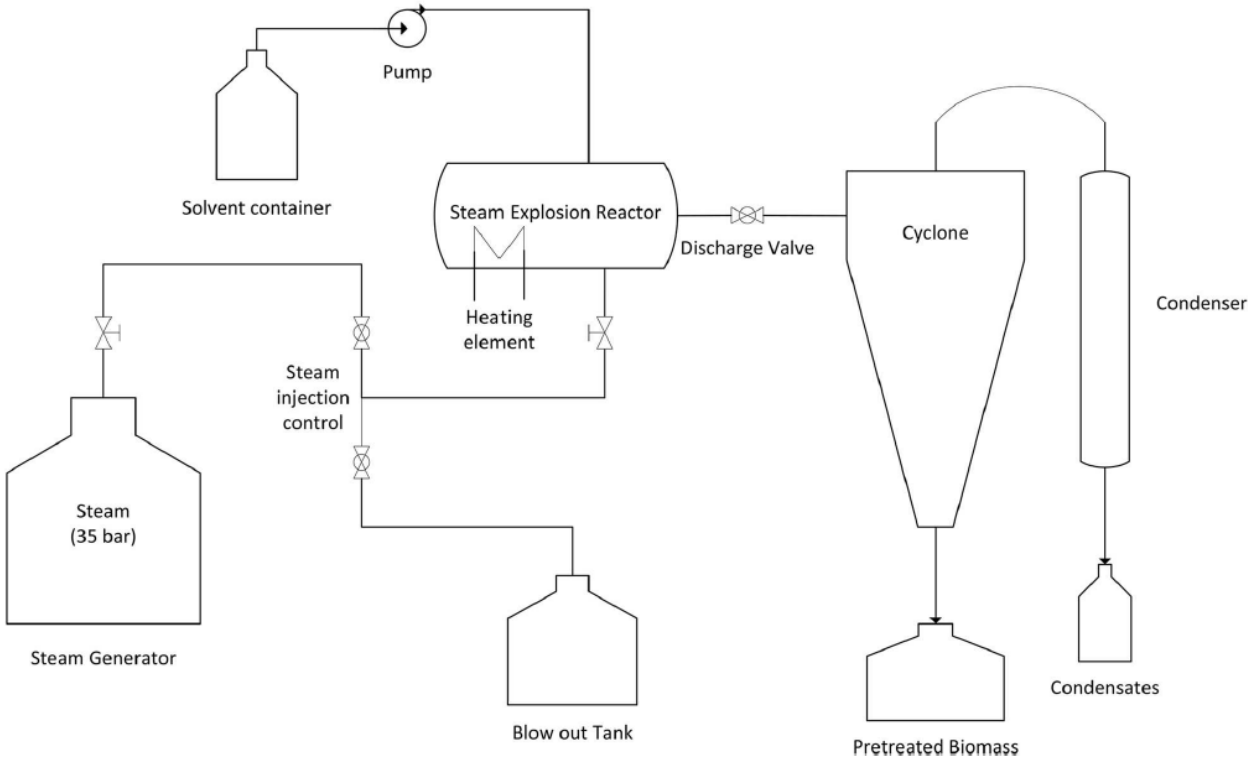

Figure 7. Schematic diagram of steam explosion and a fractionation reactor [109].

2.3.2. Autoclaving

This method is a heat transfer process that uses an absolute pressure of 0.3 MPa and a temperature of 121 °C for lipid extraction [110]. There are some studies that were conducted using a continuous reactor under a hydraulic retention time (HRT) of 15 to 20 days and at a temperature of 95 °C that show a positive energy balance. As such, it is clear there will be good biomass yield achieved by thermal pre-treatment methodologies when they are used in large-scale applications. A review of previous research studies is shown in Table 7 below.

Table 7. Overview of the previous research studies using steam explosion and autoclaving.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Steam explosion				
Nannochloropsis gaditana sp.	150 °C for 5 min	Lipid recovery 0.3–3.6%	Total lipids.	[111]
Chlorella sorokiniana	120 °C for 5 min	17.9% and 18.2% lipid extraction	Total lipids.	[108]
Scenedesmus dimorphus	100 °C–130 °C	Enchanted solubilisation	Perfect cell count, total lipids.	[112]
Botryococcus braunii	90 °C, 10 min	Hydrocarbon (0.4% at 75 °C). 97.8 wt% recovery	Biodiesel production perfect cell count, total lipids.	[113]
Scenedesmus sp.	90 °C	Efficient cell disruption	Total lipids.	[114]
Nannochloropsis	30 °C and 60 °C	Biomass yield of 41%	Total lipids.	[114]
Chlorella pyrenoidosa	130 °C for 60 min–10 mL	2.1-fold increased lipid recovery	Total lipids.	[115]
Autoclaving				
Chlorella vulgaris	100 °C for 10 min–200 mL	15.4% lipid yield	Total lipids.	[116]
	100 °C, 1.5 MPa–5 min	Lipid content of 29.34%	Total lipids.	[54]
	121 °C with 0.1 MPa for 5 min	Lipid content of 24%	Total lipids.	[117]
Botryococcus sp.	125 °C with 1.5 MPa for 5 min	5.4–11.9% lipid recovery	Total lipids.	[54]

Balancing the energy of biomass production between energy consumed during production and energy harvested in the form of fuel is essential for biomass production to be cost effective. However, some reports suggest that using thermal pre-treatments with microalgae results in a negative balance [103]. When thermal methods are compared to other pre-treatment techniques such as physical and ultrasound pre-treatment methods, the energy that is consumed is comparatively low [114].

2.4. Chemical Treatments

Chemical treatments are ways of introducing chemical substances such as alkaline or organic solvents, detergents, chaotropes, antibiotics, hypochlorites, and chelating agents to enhance cell disruption. Usually, alkaline pre-treatment methods using alkaline compounds such as potassium, sodium hydroxide, and calcium at pH levels varying from 9 to 12 are applied for algal biomass. Acid pre-treatments are carried out by exposing H_2SO_4 and HCl at lower pH levels. Antibodies have the ability to extract lipids from cell membrane components by inhibiting them from the inside, whereas chelating agents cross-couple the cell membrane molecules to cause disruption. Detergents mix with membrane molecules, with the solvents in them dissolving and piercing the cell membrane and cell wall [52,118]. Oxidising agents such as hydrogen peroxide and ozone are used to disrupt cell walls. The energy required to enhance biomass is also too low compared to other methods such as physical or thermal ones [119]. Even though chemical pre-treatment

methods are generally used for pre-treating cells, studies using them for microalgae biomass production are not as common as those using physical or thermal methods. The major problem with using chemical pre-treatments methods is that they are corrosive and toxic and may also produce inhibitory components. They may also lead to contamination [120].

Scenedesmus and *Chlorella* biomass showed pH improvements from 9 to 11 when chemical pre-treatment methods were used. By increasing the pH to 13, the microalgal cells were damaged because of the high alkalinity. It is also stated that low positive total energy values were attained in all cases [121]. Other studies concluded that treating *Chlorella* sp. *and Nannochloropsis* sp. with different alkaline solutions has a negative effect, as these microalgae have robust cell wall conditions [122]. Using oxidising agents on microalgae looks challenging when compared to acid or alkali pre-treatment methods to generate biomass. Microalgae biomass is limited when it is pre-treated by ozonation. Applying ozone pre-treatments to the biomass improved energy efficiency from 6% to 66% at different stages [123]. Apart from these chemical methods, TiO_2 and hydroxyl radicals should also be studied to see if they increase biomass energy. These methods comprise different categories: solvent pre-treatments, catalytic pre-treatments, alkali and acid treatments, and enzymatic treatment.

2.4.1. Solvent Pre-Treatment

Biochemicals such as c-phycocyanin, astaxanthin, etc., are used as solvents to extract lipids. Studies report that some amine solvents can be used for cell disruption by modifying their polarity by adding them carbon dioxide. It can be said that algal biomass is highly influenced by amine solvents [124]. Additionally, not all amine solvents react with CO_2, and interaction depends on the polar compounds in the form of algal carbamates. To study this, more studies are needed to determine the properties of the reaction. In order to control the high energy consumption needed at the time of cell disruption and drying, switchable hydrophilicity solvents (SHSs) can be used. SHSs have the capacity to extract lipids by making contact with the lipids and organic solvents, thus increasing the extraction efficiency [125]. A review of the relevant research literature is shown in Table 8.

2.4.2. Catalytic Pre-Treatment

These methods comprise heterogeneous and homogeneous catalytic pre-treatment methods for biodiesel production [126]. Studies have noted that the use of a homogeneous catalyst in the process of biodiesel production results in advantages such as product purification, the reusability and recovery of the catalyst, lower water consumption, and less energy [127]. Research using 1 wt% of homogenous catalysts such as CH_3ONa, CH_3OK, NaOH, and KOH for biodiesel production with the addition of sunflower oil at a temperature of 60 °C for 3 h resulted in a biodiesel yield of 91.22% [128]. A review of previous research studies is shown in Table 8.

2.4.3. Enzymatic Treatment

This is a biochemical method that requires a mechanical technique, has a lower energy requirement, and can cause cells to rupture to achieve effective lipid production for biodiesel [40]. Cell efficiency can be improved by this technique, as the extraction process works with cellulose, alkaline protease, sanilase, and papain [129]. The process is non-flammable, inexpensive, and inert in nature, so it is very suitable for biodiesel production [130,131]. Additionally, enzymatic pre-treatment demonstrates an advantage when using rapeseed oil during the pre-treatment process, as it can separate microalgal strains and glycerol [132]. A review of previous research studies is shown in Table 8.

Table 8. Overview of the previous research studies using solvent, catalytic, and enzymatic treatment methods.

Microalgae	Operating Conditions	Output Studied	Review	Reference
Solvent treatment				
Chlorella vulgaris	Amine solvents (dimethylbutylamine, dipropylamine, ethylbutylamine, phenethylamine, and dimethylcyclohexylamine) + culture mixed in ratio of 1:1 with CO_2 treatment–50 mL	Lipid extraction yield of 9.16%	Total lipids.	[133]
Chlorella sp.	Dimethylbenzylamine solvent, culture (1:1 ratio for 1 h extraction time)	Lipid extraction of 25.97,32 and 40.8%.	Total lipids.	[134]
Scenedesmus	Hexane: isopropanol (3:2) and solvent; culture (75:1) for 2 h extraction time.	FAMEs of 13% and total lipids, with polar FAME about 1.5% of total lipids	Biodiesel production, total lipids.	[135]
Nannochloropsis oceanica	TEPDA solvent: culture (1:4 ratio with 2 h extraction time)	98.2% lipid extraction efficiency.	Biodiesel production, total lipids.	[125]
Catalytic treatment				
Nannochloropsis sp.	Mixing 10% of Mg and Zr for 4 h at a temperature of 65 °C	Biodiesel potassium hydroxide yield of 28.0%.	Biodiesel production, total lipids.	[136]
Monoraphidium sp.	2 mL hexane and 5 mL of 20% saturated NaCl solution.	82.86% saponifiable components and 17.14% unsaponifiable components.	Total lipids.	[137]
Chlorella vulgaris	Dried in oven at 48 h at 100 °C	Lipid yield of 53.25%	Total lipids.	[138]
	220 °C, 2 h methanol per gram of biomass–8 mL	Biodiesel yield of 74.6%	Biodiesel production, total lipids.	[139]
Scenedesmus acutus	Dried in vacuum at 60 °C for 20 h.	~99 wt% hydrocarbons for biodiesel. 12.6% of extracted lipids.	Biodiesel production, total lipids.	[140]
Enzymatic treatment				
Rhodotorula glutinis	Adding glycerol, AA, and ChCl with 60 °C for 120 min, solid–liquid ratio is 1:20	Lipid yield improved by 32.1% and 54%	Total lipids.	[107]
Chlorella vulgaris	12 h hydrolysis by protease 2% (*v*/*w*) enzymes at 45 °C for 45 min and 12 h hydrolysis by cellulase (2% *v*/*w*) enzymes at 45 °C for 45 min	44% lipid yield.	Total lipids.	[64]
	Enzymatic hydrolysis was performed at pH 4.8 and 50 °C for 72 h.	1.10–1.69-fold and 85.3% hydrolysis yield.	Total lipids.	[141]
	Mixing sanilase and trypsin enzymes for hydrolysis.	30% lipid yield	Total lipids.	[142]
Nannochloropsis sp.	Enzymatic treatment at 50 °C for 30 min and a pH of 4.	90.0% lipid yield.	Total lipids.	[143]

2.5. Biological Pre-Treatment

Biological pre-treatments involve three factors: fungi, bacterial, and enzyme activity. These methods are considered to have low investment requirements, mild operating conditions, and less energy consumption and represent the best alternative to the aggressive mechanical techniques [144]. Lipases, glucanases, peptidases and glycosidases are also the most used enzyme classification methods to disrupt the cell wall. During the process, the enzymes mix inside a molecule inside the cell wall/membrane and break the bonds, resulting in cell disruption [145]. To enhance the algal biomass, an enzyme or mixture can be increased. The mixture mostly contains cellulose, starch-degrading enzymes, and hemicellulose [146]. Biological methods may be the best alternative to chemical and physical methods, as they avoid causing inhibitory problems; they are effective low-temperature alternative techniques to thermal pre-treatment methods [96]. The major disadvantage of biological methods is that they need 10–14 days, much longer than all of the other pre-treatment methods; they also need a big space to be carried out on an industrial scale. These methods can be used by themselves or can be combined with other pre-treatment techniques if the concentration of the recalcitrant compound is very high [147]. Some studies have shown that biological pre-treatment methods are mainly used for commercial enzymes and result in high methane output [148]. Biological pre-treatment methods also include algicidal pre-treatments, which consist of viruses, cyanobacteria, bacteria, and microalgae themselves. They have the capability to attack the extracellular compounds on the microalgae to extract the lipids [149]. The use of *Chlorella vulgaris* ESP-31 with bacterium *Flammeovirga yaeyamensis* for oil extraction over a 3-day-long pre-treatment process showed enzymes breakage in xylanase, amylase, and cellulase with a high lipid content of 21.5% [150]. Furthermore, a similar investigation of *Nannochloropsis* sp. biomass with different combinations of lysozyme, protease, cellulose, and pectinase enzymes showed a higher lipid content than when a single enzyme was used [143].

2.6. Supercritical Fluid Extraction

This method is said to be on the best and most effective techniques for lipid extraction processes and is eco-friendly [27,151]. This method requires the pressure and temperature to be increased more than the critical point to induce cell breakage. Substances such as CO_2, ammonia, methanol, and others can be used as supercritical extractants, and SC-CO_2 is the most commonly substance used due to its low cost, low temperature, and low pressure [152]. Lipids can be also directly transestrified into biodiesel using this method [153]. A study with the microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* in combination with ethanol as a co-solvent resulted in cell disruption and extracted lipid percentages of 97% and 83% [154]. A similar study conducted by Viguera et al. [155] using *Chlorella protothecoides* microalgal species at 70 °C and 300 bars resulted in a higher lipid yield rate. SC-CO_2 along with *n*-hexane was used for lipid extraction in the microalgae *Schizochytrium* sp., and the results suggested that the lipid efficiency extracted from SC-CO_2 was more than the efficiency obtained from *n*-hexane [156]. Another study compared Bligh–Dyer with SC-CO_2 using *Scenedesmus obtusiusculus* and *Scenedesmus obliquus* at 12 Mpa and 20 °C and found that the lipid extraction rate was higher than 90% in the SC-CO_2 process, and the author suggested that this method is good for industrial purposes [157]. Using ethanol as a co-solvent in *Pavlova lutheri.* with various operating and extraction conditions, De Melo et al. achieved 3.5-fold and 7.9-fold higher extraction than fish oil [158]. The main problem with this method is the high price of the equipment and its limitations in large-scale applications.

2.7. Pulsed Electric Treatment

Pulsed electric and high-intensity field pulsed methods are techniques that use electric fields to disrupt the cell and produce lipid extraction. This produces electro-mechanical vibrations and an electric field that creates tension in the cell wall/membrane [159].

The high-strength power of ~30 kV passes through the cell wall/membrane, and by increasing the power of the electric field by ~2000 Hz, a large number of dissimilar electric charges pass over the dipolar molecules and break large elements, decreasing the complex molecular forms and piercing into the cell [160]. When the electric field exceeds a specific voltage, the inner pressure generated within the membrane creates an unequal amount of energy in an attempt to form unrepairable pores in the cell [161]. This pulse electric not only kills all of the cells in the membranes, but also attacks the molecular components within the cell. This technique also affects the nutritional products and the proteins due to the very high temperature [130]. This method has the advantage of being combined with other methods to achieve efficient cell disruption, but the solution that is added should be ion free. When treating marine algae with this pulse effect, the microalgae need to be prewashed and deionised to improve the ability of the pulse field to pass into them. This pre-treatment method produces mixed outputs. A study showed that less than 5% biogas was produced from the biomass [160]. Due to these problems, this method is not favoured by biorefineries.

2.8. Combined Pre-Treatment Methods

Combining various pre-treatment techniques can be used to reduce costs and enhance efficiency. Thermochemical pre-treatment methods are a blend of thermal and chemical methods, and when applied to *spirulina* biomass, they showed low biogas production, as these methods use toxic and chloride-based chemicals with a low pH [84]. Research using combined processes used the microwave and bead mills pre-treatment processes along with cell shattering via high-frequency shock waves [162,163]. The sonication method breaks the cell wall and reduces the size because of the cavitation effect [164]. Cell disruption occurred during bead beating in thee microalgal cells due to the high-speed spinning beads [80,82]. The amount of energy can be reduced for lipid extraction by combining ultrasonic and chemical cell disruption methods [117]. There are fewer studies on the use of combined methods compared to those highlighting the use of single pre-treatment methods, so future studies can focus on using combining methods for cell disruption. A list of previous studies is shown in Table 9.

Table 9. Overview of the previous research studies combining pre-treatment methods.

Microalgae	Treatment Type	Operating Conditions	Production Yield	References
Chlorella sp.	Homogenisation + thermal	84 MPa (123 °C and pH of 1.5, chloroform, methanol	Efficiency of 4.5 (mg/g cell)	[165]
Chlorella vulgaris	Microwave + solvent	700 W, 50 s–chloroform:methanol:water (2:2:1.9)	Lipid recovery of 31.70	[141]
Nannochloropsis oceanica	Microwave + diluted acid	140 °C, 25 min–H_2SO_4 (1% *v*/*v*)	Hydrogen yield of 183.9 mL/g TVS	[166]
Scenedesmus sp.	Thermal + alkaline	100 °C, 8 h–NaOH	Lipid extraction-45.54 mL H_2/g (VS) of hydrogen	[167]
	Ultrasonication + solvent	30 kHz, 1 kW for 5 to 60 min–hexane, chloroform/methanol (1:1 *v*/*v*)	Efficiency of 0.144 to 0.72 (mg/g cell)	[168]

2.9. Other Latest Pre-Treatment Techniques

There is a need for novel techniques to decrease the recalcitrant properties of microalgae and influence biomass to disrupt high-value lipids, and new techniques are being implemented. Most researchers consider their work to be eco-friendly and appropriate for large-scale production. However, while their findings may be of good quality, they focus on industrial applications.

Updates to biological techniques with modifications were implemented in a recent study on lignocellulosic biomass, which showed that changing cellulose elements leads to increased enzyme hydrolysis, lower energy use, lower operational costs, and less hemicellulose loss [169,170].

The photocatalysis method uses light absorption to increase the temperature, creating a chemical reaction. *Chlorella vulgaris* treatment saw a mineralisation efficiency of 57% efficiency when using a light intensity of 4000 lux and a temperature of 25 ± 1 °C [171]. *Chlorella pyrenoidosa* and *Scenedesmus obliquus* treatment were investigated and resulted in decreased cell toxicity [171].

A study on cell disruption techniques resulted in advances in explosive decompression. This method uses propane, butane, or carbon dioxide for lipid extraction. *Haematococcus pluvialis* was suspended at a dry cell weight of 18.11%, and using explosive decompression, extraction increased from 72.3% to 92.6%. Because of the higher dry cell weight and the lower specific energy consumption, a high extraction yield was obtained [172].

Another study used pulsed arch technology in grape seeds to disrupt cells. This technology used high-electric energy discharge during a time phase and produced cavities within the cells due to the high temperature and pressure [173]. This is one among a number of highly aggressive methods, but it has still not been studied in microalgae. Perhaps this method could be modified in the future so that it could be used to disrupt cells with low electric energy, shear forces, and temperature to obtain good lipid productivity.

The autolysis extraction technique, a less explored method, represents a good approach for disrupting lipids. Cell disruption can be triggered by different atmosphere cues such as anoxia when there is an increase in temperature. *Chlamydomonas reinhardtii* [174] *Nannochloropsis gaditana* [175] were treated at temperatures of 50 °C and 38 °C, respectively, during a 24 h incubation period and showed cell breakage. This technique is considered for application because of its mild treatment conditions and low processing costs, even though this method seems to be slow.

Ionic liquid methods have been indicated to facilitate lipid extraction from wet microalgae. The influence of the ionic liquid 1-ethyl-3-methylimmidazolium methyl sulphate [EMIM][$MeSO_4$] was tested on *Nannochloropsis* sp. and resulted in a biodiesel yield of 40.9% [97]. Another study was conducted with 1 g of dry *Chlorella vulgaris* that had 4 mL of ionic liquid (1,3-dimethylimidazolium methyl phosphate) + 4 mL of methanol added to it, and it was treated for about 18 h at 65 °C. The results indicated the occurrence of cell disruption [176]. This method is also considered to be a mild process with slow results.

Research was conducted with *Nannochloropsis oculate* and *Scenedesmus dimorphus* using convectional ultrasonication at 100 W and a 20 kHz frequency and focused ultrasonication at 40 W and 3.2 MHz. The results indicated that higher efficiency and a better cell disruption efficiency can be obtained using focused ultrasonication than with convectional ultrasonication [177].

Pressurised liquid extraction is a technique that is also known as an accelerated solvent extraction technique. This method extracts the intercellular compounds in a shorter extraction time by utilizing a combination of pressure and temperature [178]. In addition, there have been several studies that have used supercritical water, propane, dimethyl ether, and n- butane as solvents [178,179].

3. Comparison and Discussion on Different Pre-Treatment Techniques

Microalgae are single-cell organisms. Their photosynthesis produces around 70% of the O_2 in the atmosphere. Not all microalgae are single-cell organisms, and some species of microalgae can grow as single cells or in colonies (according to colour) and take the shape of spheres or filaments [180]. During cell disruption, the inner forces cause temperature changes as well as cavitation, pressure, and molecular energy variations. The quality of the final product may be impacted by these events separately or collectively, depending on whether the contaminants are produced or the algal elements are degraded [181]. From the above methods, it is clear that mechanical and physical methods are considered to be

more effective in enhancing lipid efficiency in large-scale applications. The cell disruption caused during high-speed homogenisation and bead milling works according to the principle of shear force, which is similar to the energy transfer caused by the current and waves effects observed in the microwave, ultrasound, and pulsed electric field techniques. Enhancing the efficiency of microalgae mainly relies on cell wall characteristics as well as on strain and operational parameters, including temperature, enzyme doses, and power input. Often, pre-treatment is the best way to improve biomass production with various ranges of efficiencies. Studies suggest that biomass yield can improve from 20% to 60% after the application of pre-treatment methods. Figure 8 shows the various microalgae that have been studied using different pre-treatment methods. Using a thermal pre-treatment method, *Botryococcus* sp. showed an improved lipid yield and increased biomass production [54]. The homogeniser and microwave methods were found to be more efficient compared to other methods. *Botryococcus* sp. showed lipid production of 28.6% and 28.1% when the microwave and homogeniser methods were used, respectively, and the bead beating method showed a higher lipid percentage when used with *Botryococcus braunii* compared to other pre-treatment techniques such as the French press, sonication, and homogeniser methods [164]. It should also be noted that the efficiency of the bead beating technique is not easily measured. *Chlorella vulgaris* showed high efficiency when the microwave method was applied and 7.9% lower efficiency compared to other mechanical methods. The microwave pre-treatment of *Scenedesmus* sp. resulted in high lipid efficiency when compared to other methods. However, the osmotic pre-treatment method is quite simple and produced similar output to the mechanical methods for *Scenedesmus* sp. and *Chlorella vulgaris*. A small problem with this method is that it requires a long pre-treatment time of 48 h [54]. A pre-treatment method similar to the microwave method that uses animal fats and vegetable oils has been studied and suggests that the microwave method is a simple, efficient, and easy pre-treatment technique. Furthermore, this research indicated the lipid extraction can be also easily measured and concluded that the microwave technique is the most applicable method for the large-scale production of microalgal biomass [163,182].

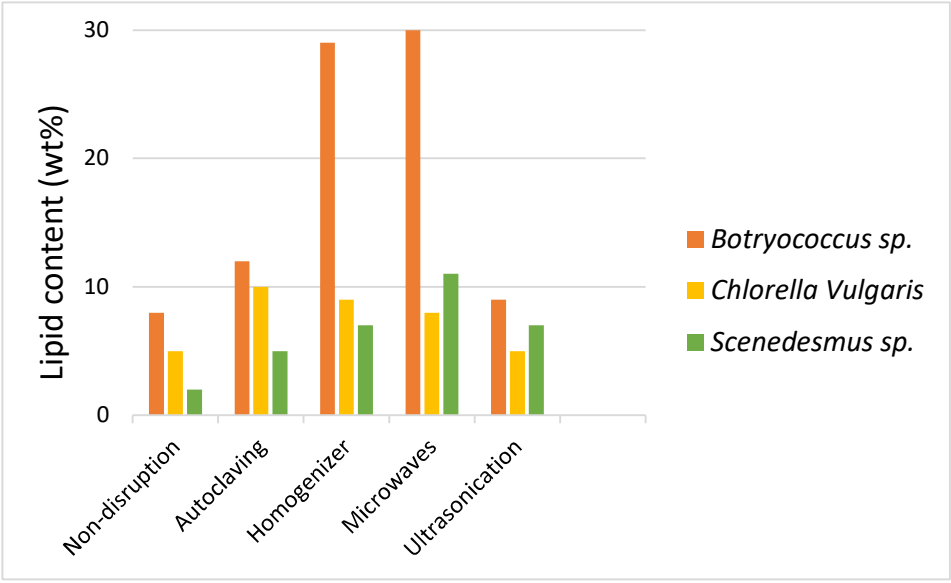

Figure 8. Schematic representation of lipid productivity of microalgae [54].

In various studies, modifying the pre-treatment methods and conditions has been seen as a strategy to enhance lipid production. The current studies in microalgal biofuel are mentioned because they highlight the ability of different methods to recover lipids [183]. The biomass production and the lipid recovery rate change from species to species

and also vary across treatment methodologies. *Dunaliella salina* and *chaeoceros muelleri* were evaluated using osmotic shock by Lina et al. [184], where the effect of biomass and the water ratio was analysed with different levels of fluorescence ranges and timings. Many results were generated with different iterations, and it was concluded that the various results differ according to economic and efficiency factors. In a work by Gruber et al. [185], studies were conducted with methods such as microwave, ultrasonication, enzymatic, and wet milling using the microalgal biomass of *Chlorella vulgaris, Acutodesmus obliquus,* and *Chlorella emersonii* to study single and combined pre-treatment technique effects, and varying lipid-recovery rates and cost analysis were achieved. Another study by Francesso et al. [186] looked over the impacts of cell rupture using thermal, thermal hydrolysis, enzymatic, and ultrasound techniques using *Scenedesmus obliquus* and *Chlorella vulgaris*. In the initial set of experiments, the yields were shown to increase with the ultrasonic and enzymatic techniques compared to the untreated biomass, and then, when combining the thermal and thermal hydrolysis strategies, the yield percentages were lower. Additionally, apart from modifying and combining pre-treatment techniques, most of the studies focussed on cultivation process such as the selection of species [187], growth media [188], CO_2 [189], light [190], temperature [191], and nutrients [192]. Hence, the first suggestion for the microalgal pre-treatment is to determine the effects of the processing conditions, modifications, and pre-treatment techniques in combination to obtain an increase in the yield percentage functions. In a previous report using microalgae *Botryococcus braunii* pre-treated with thermal method for 140 °C for 10 min, a recovered lipid yield of 97.8 wt% was obtained [113]. In research using chemical methods and *Nannochloropsis oceanica* with diluted H_2SO_4 (1% *v*/*v*), the researcher obtained a hydrogen yield of 183.9 mL/g TVS [167]. Thermal pre-treatments are recommended compared to other methods such as ultrasound and biological pre-treatment methods [80]. For *Chlorella vulgaris*, enzymatic pre-treatment methods were more effective in enhancing biomass production. It also represents an energy-balanced pre-treatment method other than hydrothermal, thermal, and ultra-sound treatments. When thermal, ultrasound, hydrothermal, and microwave pre-treatments were compared using microalgae obtained from an open pond, a high lipid yield, organic matter solubilisation, and biomass concentration were found. It was also noted that thermal pre-treatment methods showed a positive energy balance because of energy gain [193]. Physical pre-treatment methods using ultrasound or microwaves are used to increase the biomass. The final quality of the product is related to the biochemical composition and morphology of the microalgae during cell disruption. The cell disruption effect was determined by Komaki et al. [194] by studying three various strains of *chlorella vulgaris*. The results indicated that digestibility occurred in one among them. Additionally, the selection of the extraction process has an impact on the final product. The summary of advantages and disadvantages of various pre-treatment techniques are shown in Table 10.

Table 10. The summary of advantages and limitations of various pre-treatment techniques.

Cell rupture Method	Parameters Affecting Lipid Production	Advantages	Disadvantages	Reference
Mechanical	Design of the blade, number of passes, pressure, and speed of rotation.	Surface area increases. No inhibitory or toxic compounds. Easy to operate and commercialisable. Biomass is easy to handle.	Requires high energy. High capital and maintenance costs. Influence inert materials.	[25,195]
Ultrasonic	Power, time, and cycle number.	Extraction time and solvent consumption are reduced. Bulk medium of cell contents is reduced.	Requires high energy consumption. Scaling up is difficult.	[90,196–198]

		No inhibitory or toxic compounds.	High capital and maintenance costs.	
Microwave	Temperature, stirring, power, and time.	Less energy demand and solvent usage. Fast and uniform heating. Eco-friendly. High extraction yield.	Efficiency differs when solvents are volatile or nonpolar. Scaling up is difficult.	[163,199]
Autoclaving	Temperature, thermal stress, and pressure.	High lipid content. Life span of the product of maintained. Lower energy demands.	Requires high energy consumption for industrial processes. Time consuming process and scaling up is difficult.	[25,200,201]
Steam Explosion	Temperature, thermal stress, microalgae species, and pressure.	No inhibitory or toxic compounds. Hazardous wastes can be reduced during lipid recovery. Low cost and commercialisable.	Efficiency is dependent on microalgae species. Requires high energy consumption for industrial processes. Time consuming process and scaling up is difficult. Energy costs for high temperatures.	[111,202,203]
Catalytic	Stirring, chemical concentration of KOH and NaOH.	Lower energy consumption. Hemicellulose solubilisation.	Expensive chemical cost. Toxic and inhibitory. Contamination during extraction.	[8,118]
Enzymatic	Enzymatic type, stirring.	Lower energy consumption. Higher lipid yield and speed process.	Expensive enzymatic cost. Agitation conditions.	[204,205]
Pulsed electric field treatment	Oscillation, time, microalgae type, growth phase conditions, and conductivity.	High lipid content. Non-inhibitory compounds. Speed and uniform cell disruption.	Requires high energy consumption for industrial process. High capital and maintenance costs.	[206]
Biological	Enzymes and combination of enzymes.	Energy demand is low. Non-inhibitory compounds.	Cross-contamination. High enzyme cost. Requires large space. Slow pre-treatment process.	[207]

For the process of biodiesel production, cell disruption and lipid extraction from microalgae have been studied and researched for years. The cell disruption pre-treatment techniques used for microalgae have both pros and cons depending on the energy con-

sumption, type of microalgae, cost effectiveness, applicability, and efficiency. As discussed above, further advanced studies may be required to face the challenges of these methods and to bring them to reality.

4. Selection and Processing of Pre-Treatment Technique

The selection of a pre-treatment method and other processing steps is very crucial. These steps must be followed throughout the research process, from microalgae culturing to final processing. There are numerous choices for processing, and some steps may favour the discovery of new techniques.

1. Many iterations must be carried out for process development using published literature. New methods should be evaluated and analysed for lipid yield by considering the environmental impacts and cost factors of the method.
2. Improvement must be carried out according to successful studies using the available modern techniques, and the processing should be continued.
3. Energy and cost are very significant. Along with this, product evaluation is also important and can show an increase in process profitability.
4. Following the safety and legislation protocols is also essential. When using chemical methods, it is very important to consider safety and legislation factors.

5. Energy Consumption of Pre-Treatment Techniques

Even if pre-treatment techniques increase recovery, they demand a certain amount of energy for processing. The evaluation of cost effectiveness and energy consumption of different pre-treatment techniques should be economically relevant. The energy requirements should be viewed according to several factors, such as type of species, growth conditions, pre-treatment method, concentration, etc. For the mechanical and physical methods, the energy demands are ultimately high compared to other methods. Generally, for these methods, the energy consumption and cost effectiveness are the most influential conditions. However, for non-mechanical methods, the energy consumption depends on stirring, time, and temperature. Compared to biological enzymatic hydrolysis treatments, this technique requires very less energy but is only dependent on stirring [208]. By working on the industrial conditions, this method can be optimised in terms of its operating parameters to have a shorter working duration, which might increase its cost as well [209]. Compared the various mechanical methods such as the high-pressure homogenisation (HPH), high-speed homogenisation, microwave, ultrasonic, and bead milling techniques, studies conclude that HPH seems to be a more energy consuming technique [52–54] followed by the ultrasonication and microwave techniques [210]. Therefore, further studies have to be carried out to predict an actual energy demand that will help the specific pre-treatment technique chosen for industrial applications.

6. Key Challenges and Future Perspectives

Current applications in biofuel industries demand new economically and environmentally sustainable processes to overcome the demerits that they face. As such, large-scale and effective techniques for microalgal lipid extraction are required, and a considerable number of studies are needed. Future research should be focused on decreasing the energy consumption, overall cost effectiveness, adaptability, mildness, and recoverability of bio-products. As such, it is important to intensify approaches to minimise the cost and to utilise microalgal extracts to their full extent. In some species, genetic modifications that change their microbiological characteristics may pave a way to introduce new strains with enhanced outputs. Another challenge lies in contamination: it is better to carefully control the development of microorganisms such as bacteria, virus, and other predators. These can become a danger in growing cultures (affecting their growth) and might also reduce the efficiency [149,211]. As such, it is better to look for a potential new route to sort out

this complex issue. Radio frequencies using certain magnetrons might cause rapid thermal effects and agitation, but this process has not been addressed in depth. However, a study on the non-thermal effects obtained using microwave radiation gives us a small idea about this process [212]. Pre-treatment techniques such as ultrasonic, microwave, and pulsed effect methods are currently being researched in combination with new approaches on a small scale, but if it possible to control their energy consumption, these methods could be used in large-scale industrial applications. A recent popular technique is the pulsed electric field technique, but this technique still being researched. The creation of new models and improving flow techniques are challenging to investigate. The HPH and bead mill methods are the most effective cell disruption techniques and are able to handle robust cell walls. However, cell walls are diverse, nano-biotechnology research may be promising for processing the weak cells. Alternatively, combining mechanical and non-mechanical methods, e.g., ultrasonic methods with enzymatic or chemical methods, could reduce energy demands. Studies are required for up-and-coming methods such as explosive decompression and cationic polymer-coated membrane treatment to obtain their cell disruption ability for lipid extraction. A method for collecting generic data about different lipid efficiencies should be produced, this could help us to achieve higher level of understanding and would encourage researchers to discover new mild techniques.

7. Conclusions

Considering the pre-treatment techniques reviewed in this paper, the studies shown here attempt to provide cost-effective solutions for the increasing universal energy demands. Complex cell wall/membrane structures may remain as an obstacle to biodiesel production; however, the application of pre-treatment methods will improve the quality of feedstock yields by disrupting the microalgal cells and extracting lipids. Depending on the type of microalgal species, the cell wall/membrane structure acts as a main factor that influences microalgae solubilisation and has various efficiency outcomes. Although most of the pre-treatment techniques were found to have positive attributes in biodiesel production, after checking numerous ongoing research studies, it is well-understood that there is no best methodology for the application of pre-techniques for microalgal lipid extraction because each and every method has both pros and cons for different microalgal species, and the lipid productivity percentage relies on the microalgal species being considered and their characteristics during cell rupture. In large-scale applications, energy and cost requirements are denoted as main indicators, and the discussed techniques are not always feasible for biorefineries due to the high energy consumption and operational costs. The major aspects of industrial microalgae lipid extraction techniques are their universality, energy efficiency, selectivity, mildness, and controllability. Recent research shows that mechanical techniques are optimal for industrial lipid extraction, but they consume a large amount of energy. Non-mechanical techniques may have a low energy demand, but the final quality may be low, and they have a long pre-treatment time. Studies related to biological pre-treatment during on-site enzyme production and enzyme immobilisation indicate that the cost of pure enzymes is high and non-recyclable. Therefore, further studies must focus on reducing the cost of biological pre-treatment methods. Thermal, biological, and chemical pre-treatment methods have been found to produce a higher energy balance than microwave and ultrasonic methods. Microwave and ultrasonic methods lack in biomass yield enhancement and are easily commercialisable. Capital investment in ultrasound and microwave pre-treatment methods is considerably higher than investment in biological and chemical pre-treatment methods. Energy-demanding techniques such as the bead mill or high-pressure homogeniser techniques are mostly preferred for large-scale applications. Future studies can place importance on oxidative pre-treatment techniques as well pulsed and laser electric arc techniques for cell disruption technologies for biodiesel production. These can be very useful for increasing the potential

capabilities of microalgal biomass. Hence, it can be concluded that pre-treatment techniques must aim to provide an enhanced lipid efficiency with a reduced energy demand to produce a sustainable energy source.

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