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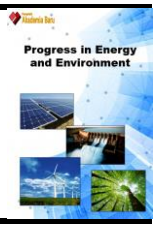

Techniques of Improving Microalgae in Biomass Clean Energy: A Short Review

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ABSTRACT

Microalgae have been considered as a reliable feedstock for biodiesel production and regarded as the promising alternative source to replace petroleum-based fuels. Production of biofuel derived from microalgae undergoes several processes, including cultivation, harvesting, extraction and biofuel conversion. However, recovery of intracellular content is time-consuming and difficult process as the biodegradability of microalgae is strictly hindered by the rigid nature of the cell wall. Hence, pretreatment of microalgae becomes an inevitable process to facilitate cell wall disruption and liberation of organic cell contents for the biofuel production. This paper aims to review and compare the various pretreatment methods (mechanical and non-mechanical) of microalgae with respect of their strength and limitation.

1. Introduction

The rapid population growth has caused an increase of global energy demand. The global consumption and production of oil, natural gas and coal remained as the strongest growth rates over the past few years [1]. Extensive consumption of fossil fuel has led to detrimental environmental consequences of greenhouse gases and eventual global climate change [2]. However, the fact that fossil fuels are non-renewable source of energy and the forthcoming depletion of fossil fuel reserves has raise the global attention towards the search of alternative energy substitution [3]. One of the potential substitutions is replacing fossil fuels with biofuel. Biofuels refer to liquid or gaseous fuel for the transport sector produced from biomass. They are known to be environmentally friendly as they could be considered as a part of the carbon dioxide-cycle in combustion. Biofuel contributes sustainability by recycling the carbon during the biological processes, resulting in no net releases of carbon dioxide and has very low sulphur content [4]. Different type of biomass can be utilised to produce different kinds of biofuels depending on its content. For instance, oil extracted from soybean and sunflower consists content of fatty acid (m)ethyl esters which are also the main constituent of biodiesel. It is also

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usable when mixed with diesel fuel. Meanwhile, wheat and potatoes with rich carbohydrates (hemicellulose and cellulose) content can be converted into bioethanol through hydrolysis and fermentation process [5].

In recent years, microalgae has been gaining much attention as a biomass with high biofuel production potential including biodiesel, bioethanol, methane gas, hydrogen gas, etc. [6]. The ability to grow in both saline and fresh water, and the capability of rapid growth that produces high biomass yield make microalgae an excellent choice for biofuel production [7]. Furthermore, microalgae feedstock is economically and sustainability attractive as it has potential to provide high yield source of biofuel without competing with food supplies and helps mitigation of atmospheric carbon dioxide. Microalgae does not require large fertile land for cultivation [6]. Besides, technology nowadays is shifting towards algal carbohydrates content as potential feedstock for bioethanol production [6].

Few series of processes are required to convert microalgae to biofuel, which can be categorised as cultivation, harvesting, pretreatment and conversion to biofuel. Cultivation is the process where microalgae are artificially cultivated using either natural (e.g. lake, pond) or artificial (e.g. photobioreactor, raceway pond) medium. Generally, the optimal cultivation temperature for microalgae is around 20 to 30^{o}C [8]. Microalgae requires carbon source and solar radiation as the main sources to facilitate photosynthesis reaction. There are two types of microalgae cultivations system, which classified as open or closed system. For open system, these microalgae are cultivated in open environment. Meanwhile in closed system, vessel with transparent wall is used to cultivate the microalgae and allows it to expose under the sunlight. Other modified cultivation methods with better efficiency and system sustainability are photo-bioreactors (PBR) and hybrid photo-bioreactors, but these methods consume higher maintenance cost [6]. Microalgae is subjected to harvesting after cultivation where diluted microalgae suspension are being concentrated into thick microalgae aggregates and separated from the bulk culture. Microalgae cells carry negative charge that prevents itself from self-aggregate in suspension [6]. Economical microalgae harvesting is a challenging process, as it required extensive energy input to remove large amount of water in order to obtain concentrated biomass. This is due to the nature tiny size of microalgae and low cell densities in the culture medium [9]. Different studies showed that the harvesting step usually accounts about 20 to 30% of the total production cost of microalgae biodiesel [3,6,9]. Hence, harvesting of microalgae culture often regarded as one of the major economic concern that affect large-scale microalgae biodiesel production [9]. Moreover, selecting appropriate harvesting methods is strongly relies on the condition and characteristic of microalgae species and the specification of desired final product. Different type of microalgae with different contents will also affect the pretreatment and conversion method at the later stage. For instance, flocculation is one of the harvesting methods to aggregate dispersed negatively charged microalgae. There are few types of flocculants, organic and inorganic flocculants, which help neutralizing the microalgae surface charge. The effectiveness of inorganic flocculants increases with the increased in charge density [3]. When inorganic flocculants is used for large scale microalgae harvesting, large dosage of flocculants are required, causes the production of high quantity of sludge and may contaminate the yielded biomass [10]. Meanwhile, when organic flocculants is in used for the same purpose, relatively lower dosage of flocculants are required, thus reducing the amount of undesirable contamination [2]. The efficiency of organic flocculants relies on the microalgae pH level and biomass concentration of algae culture. Hence, harvesting methods would require more research in order to create a more effective process [6,11–15].

There are many factors that affect the quality and amount of biofuel produced, including the cultivation, harvesting and pretreatment conditions and quality of the microalgae. Traits that define the quality of microalgae are their capability to be produced in large quantity at low cost and the amount of contents in the microalgae, e.g. lipid, fatty acid, water content etc. [16]. Despite the various benefits associated with the production of biodiesel using microalgae, researchers are facing many technical obstacles, such as complex processes, high maintenance and production costs, production duration and high demand of energy input for conversion. For this reason, pretreatment of microalgae is crucial in

the biofuel production process. With the advancement of various microalgae pretreatment technologies, it helps in reducing the bottleneck effect of the chain process by improving the reaction rate.

After harvesting the microalgae, the microalgae will be subjected to pretreatment. Pretreatment is the process where the microalgae cell walls are ruptured to degrade the microalgae cell walls as a mean to access their content, this process can be done in both mechanical and non-mechanical way.

Cell disruption using mechanical technique are less dependent on microalgae species and less likely to contaminate the intracellular content of microalgae [17]. Most mechanical pretreatment methods, such as high pressure homogenizer, microwave, bead milling and ultrasonication, utilize physical forces to disrupt the microbial cell wall or to make the pieces of substrate smaller, squeeze them to break open the cellular structure, increasing the specific surface area of the biomass [18]. Non-mechanical pretreatment methods often involve chemical agents or enzymatic addition. Among chemical methods, acid and alkali reagents are commonly used to solubilize the hemicellulose and lignin presented in microalgae biomass, making them open for enzymatic attacks [19]. Also, enzymatic pretreatment able to target specifically parts of the cells, which make it possible to mildly disrupt the cell walls. Enzymes can be used to target a specific chemical bonding of the cell wall and therefore does not affect the contents [19,20].

In this paper, we will discuss about the various microalgae pretreatment methods. The cell disruption mechanism and process parameters vary with each different pretreatment method. In general, these pretreatment operation were categorized into two main groups: mechanical and non-mechanical pretreatment. Recovery of intracellular content of microalgae is challenging as the nature structure of cell wall hinders the anaerobic digestion to take place. The full-scale process and development consume a lot of energy during lipid extraction process. Thus, this problem becomes the production bottleneck that inhibit industrial process efficiency [19]. Despite these challenges, efficient cell disruption becomes an essential pretreatment process to capitalize product recovery from the microalgae biomass. Pretreatment of microalgae often aims to allow faster anaerobic digestion rate, potentially increase biofuel yield and prevent processing problems such as high electricity requirements for formation of floating layers [18]. The main objective is to review these existing pretreatment methods and understand the strength and limitation of each methods. At last, these pretreatment methods are discussed and compared according to few important aspects including the feasibility in large quantity, biofuel production, energy consumption, duration and estimated cost of pretreatment and sustainability. The study of comparison of various pretreatment method is challenging due to the wide range of different technologies, operating condition and information from different providers, thus detail information such as pretreatment operating cost is not discussed in this study.

2. Mechanical Pretreatment

Mechanical pretreatment utilises physical forces such as shear forces, waves, currents, or heat to disrupt the microalgae cell walls. It is performed by physically puncturing or tearing the cell walls, which makes them a harsher approach compare to the gentler chemical cell disruption methods. Besides, mechanical methods are suitable for a larger variant of microalgae species and has lower probability of contamination compared to chemical approach [9,21].

2.1 *Bead Milling*

Bead milling is a type of mechanical cell disruption methods that uses small glass or ceramic beads to puncture or damage microalgae cell walls through high speed collision. There are two types of bead milling machines, which are shaking vessels and agitated beads. The difference between shaking vessels and agitated beads machine is that shaking vessels can cause cell disruption by shaking the

vessel in perpetual motion while agitated beads is rotating the agitator inside a fixed vessels filled with beads [19]. Bead milling is sometimes preferred as it rarely causes contamination, and the bead can be easily separated by gravity from the solution [20].

Size and density of the bead, biomass concentration, microalgae species, agitation speed, and operation duration are the main factors that affect the effectiveness of the bead milling process. Additionally, optimal diameter of the beads size for effective microalgae disruption is 0.5 mm and higher agitation speed has shown positive effect on the effectiveness [20,21]. This can be explained as the fact that smaller beads and high velocity conditions increase the stress frequency significantly, whereas large beads (e.g., 1.3mm diameter beads) show no improvement in cell disruption efficiency when the agitation speed is increased [22]. Furthermore, high density beads made of zirconium or titanium carbide showed better functionality against biomass with high viscosity [21,23]. Studies suggested that the optimal energy efficient cell disruption can be achieved when biomass are fixed at concentrations of 100 to 200 g/L [20,23]. Increasing the treatment time, agitation speed and number of cycles have positive effect on the degree of cell disruption. However, prolonging treatment duration and increasing the number of cycles certainly increases the specific energy demand (kWh/kg) and greatly increasing the cost of pretreatment [21]. Hence, the condition and specific energy input of bead milling pretreatment need to be carefully selected to maximize lipid yield and achieve optimization of the energy for cell disruption.

The rate of lipid recovery from different microalgae species under bead milling pretreatment is tabulated in Table 1 below. Experiment performed on *Chlorella vulgaris* species has shown that by increasing the operation time, an increase of content released by the microalgae has been recorded, where 90% – 95% of cells are disintegrated after an operation time of 200s to 250s [24]. Another example, Lee et al. disrupted 100 mL suspensions (mass concentration 5 kg m^{-3}) of the microalgae *Botryococcus*, *Chlorella* and *Scenedesmus*, by bead mill with an energy input of 840 W for 5 minute, this energy consumption is equivalent to 504MJ kg^{-1} of dry mass [23].

Table 1 Summary of bead milling pretreatment on different microalgae species

Microalgae species	Condition	Lipid recovery/ efficient/ outcome	References
Chlorella sp.	7.5 kW, 0.5 mm ZrO_2 beads, 70% beads filling, 15.8% DCW, 90min	98.5% cell disintegration of Chlorella	[21]
	25 kW, 0.6–0.8 mm ZrO2 beads, 85% beads filling, 12.4% DCW	85.29% cell disintegration of Chlorella	
Botryococcus sp.	Bead beater, 840W, 0.1mm, 2800rpm, 5min.	20% increment in lipid recovery	[23,25]
Chlorella vulgaris		3% increment in lipid recovery	
Scenedesmus sp.		6% increment in lipid recovery	
Chlorella vulgaris	1mm ZrO_2 beads, 65% bead filling, 200-250s	90-95% cell disintegration	[24]

From the table 1, the efficiency of lipid extraction from microalgae varies with the species. Among the different microalgae species under bead milling pretreatment, the highest increment in lipid recovery is *Botryococcus sp.* as the nature lipid content from *Botryococcus sp.* is relatively higher, about 160.3 mg L^{-1}. However, considerable amount of research is still needed for better understanding in the relationship between cell wall characteristics of different microalgae species and disruption efficiencies [21].

Although bead milling machine are ideal for most cases as they are suitable to be used for most microalgae species due to its mechanically operated nature. Bead milling machine usually consumes more energy than chemical methods. Moreover, increasing the amount of biomass will need to be compensated by either increasing the size of the machine or increase the amount of machine used. Therefore, bead milling machine can be used to produce biomass sample for study purposes, but would be less ideal for large biomass production as this technique consumes large amounts of energy when applying at large scale [20].

2.2 *High-pressure Homogeniser*

Apart from bead milling, a more recent mechanical pretreatment which involved the use of high-pressure homogeniser is widely explored [26,27]. High-pressure homogenizers force a stream of microalgae biomass through a very narrow orifice discharge valve, reducing the particle sizes of microalgae species within it. When microalgae biomass is passed through the homogenization cell under high pressure condition, it can cause the physical disruption of the cell wall and membrane by multiple forces, including intra-material shear force, turbulence, elongation and cavitation. High-pressure homogeniser can be performed on microalgae with high humidity percentage, which is different with other methods that only work with dry microalgae biomass. Hence, this reduces the time and energy required to dry the microalgae biomass and significantly increases the production efficiency [28]. Harvested microalgae biomass is forced through a micrometric disruption chamber under a pressure of typically around 150 MPa but it may go as high as 400 MPa [19]. High pressure forces the microalgae biomass to discharge at the restricted outlet nozzle from the chamber, creating a high-velocity jet, where the velocity increases rapidly, and the pressure decreases to atmospheric condition. At this point, it induces an intense fluid-mechanical shear force on the cell walls and therefore causing cell disruption. This process is very effective but would cause uncontrolled release of other intracellular compounds, and possibly cause degradation of compounds [19,26]. Increasing the number of passes of microalgae biomass through the high frequency homogeniser results in higher degree of cell disruption and incremental amount of lipids recovery. Similarly, an increase of pressure intensity would also produce positive effect [21,28,29]. Due to its more aggressive nature cell disruption mechanism, extreme condition of rapidly increased in temperature of microalgae biomass and intense turbulence will occur during treatment, which result in degradation of lipid contents and other intracellular metabolites. Therefore this method can only be used when the quality of the biomass is not the highest priority [21].

2.3 *Ultrasonication*

In recent works, ultrasonication has gain wide attention due to the simpler operating mechanism and condition for microalgae pretreatment. The advantage of ultrasonication is able to perform microalgae cell disruption efficiently at relatively low temperature, thus leading to less thermal protein denaturation and retaining the natural content within the cell during the lipid recovery process.

Ultrasonication is the process where high frequency acoustic wave is induced into a liquid medium generated by transducer, initiating the formation and subsequent collapse of microbubbles in the liquid under the irradiation of intense ultrasonic wave. Cavitation in the medium is caused by the cycle of oscillating ultrasound, which consist of rapid compression and decompression of sonic waves. As the

microbubbles continue to expand and contract, eventually become unstable and implodes violently. The implosion of the microbubbles under high compression triggers propagating shockwaves through the medium which in turn induce shear forces that cause cell disruption [19,21,30,31].

These ultrasonic waves are typically higher than 15 kHz – 20 kHz. Sonic wave under 50 kHz are considered low frequency while above 50 kHz are high frequency. Lower frequency wave produces more intense mechanical shear force due to the longer expansion phase of a bubble while higher frequency wave creates free radical[17,32]. However, intense cavitation can induce free radicals that will negatively impact the pretreatment process by reacting with the lipids to produce lipid hydroperoxides which is an undesired by-product [30]. The duration where the biomass is subdued to ultrasonic waves can also affect the effectiveness of the pretreatment. The longer the duration of ultrasonication, higher the energy input. Therefore, by increasing the duration of ultrasonication the pretreatment effectiveness will also be increased [30]. According to the study of effect of sonication energy on cell breakage by J. Gerde, 2015, the experiment concluded that the pretreatment for cell disruption efficiency can be improved by increasing the energy dissipated during the ultrasonic treatments [26]. The magnitude of ultrasonic energy dissipated was calculated by measuring the ultrasound pressure amplitude and time treatment duration. However, longer ultrasonication duration would increase the chance of free radical formations, this effect has been shown to be more effective at certain amplitude [30]. Unlike other mechanical methods, ultrasonication can be configured for mild disruption to cause less cell degradation, but at the cost of lower effectiveness. Table 2 shows the condition and methane recovery of ultrasonic pretreatment on different microalgae species.

Table 2 Summary of ultrasonication pretreatment on different microalgae species

Microalgae species	Condition	Methane productivity after pretreatment (mL CH_4 g VS^{-1})	Lipid recovery/ efficient/ outcome	References
Chlorella sp. and Scenedesmus sp.	180s, 130W, Ultrasonicator (VCX 130, Vibra-Cell)	385	15% increment in methane yield	[33]
Nannochloropsis salina	Cell suspension was disrupted 3 times for 45 seconds, 200W, 30kHz output (Sonifier 250, Branson)	274	21% decreased in biogas production, due to disrupted cell changed in chemical composition of culture media	[34]
Botryococcus sp.	0.5% DCW, sonicator (Sonic and Materials Inc., USA) at a resonance of 10 kHz for 5 min	-	8.8wt% increment in lipid recovery	[25]
Nannochloropsis. gaditana	Ultrasonic bath (Fisher Scientific, UK) at a resonance of 37 kHz, ambient temperature, 5 min	-	10.8wt% increment in lipid recovery	[35]

2.4 *Microwave*

Like ultrasonic pretreatment, microwaves pretreatment also demonstrated as an effective physical cell disintegration technique. Microwaves are electromagnetic waves varying from 300 MHz – 300 GHz in frequency, but generally waves that are used to carry and transmit energy are set to be 2450 MHz [21]. Microwaves are used to target and heat up specific dielectric or polar molecules through molecular friction induced by the oscillating electric field. Water are one of the dielectric molecules

that can interact with these waves and heat up in the process, when this effects is applied to microalgae cells, the heat and pressured together with the microwaves induces damage on the cell wall and therefore causing cell disruption [17,19,21]. Microwave pretreatment is one of the easiest pretreatment method to operate but it consumes more power compared to other mechanical methods [21,36]. Miri Koberg et al. had performed an experiment using modified household microwaves oven to automatically stir the biomass, therefore only simple equipment is needed to perform microwave pretreatment [37]. An overview of case studies of microwave pretreatment is presented in Table 3. Among these various microalgae species, *Botryococcus sp.* has the highest yield of lipid extraction under microwave pretreatment. Two variables can be manipulated to control the yield of the cell disruption: the power and the exposure time of the microalgae to the microwaves. Increase in either power or exposure time will increase the effectiveness of cell disruption, but at the cost of higher input power [17]. Moreover, microalgae biomass usually contains only small fraction of water, which also reduces the efficiency of the energy transmitted through the energy wave.

However, there is some drawback of microwave pretreatment. This technique is limited to polar solvents and not suitable for volatile target compounds due to the heat generation throughout the process. The formation of free radicals, temperature increase and chemical conversion could interfere with the recuperation of fragile functional compound. Similar to other mechanical methods, microwave pretreatment could cause degradation of the cells and therefore is unsuitable for mild cell disruption [19,21]

Table 3 Summary of microwave pretreatment on different microalgae species

Microalgae species	Condition	Methane productivity after pretreatment (mL CH_4 g VS^{-1})	Lipid recovery/ efficiency/outcome	References
Nannochloropsis salina	5 times until boiling at 600 W and 2450 MHz; Inverter Grill, Panasonic	487	40% increment in spec. biogas production	[34]
Botryococcus sp.	100 ºC, 2450 MHz for 5 min	-	20.6 wt.% increment in lipid extraction	[25]
Chlorella vulgaris			5.5 wt.% increment in lipid extraction	
Scenedesmus sp.			8 wt.% increment in lipid extraction	
Microalgal-bacterial biomass grown in the High rate algal pond (HRAP)	900 W output power, 3 min of exposure time (110,200 kJ/kg VS applied specific energy)	0.20 L CH_4/L	60% increment in methane yield at 20 days with hydraulic retention time (HRT)	[17]
Nannochloropsis. gaditana	2450 MHz ,150°C, 5 min	-	3% wt. increment in lipid recovery	[35]

2.5 *Steam Explosion*

Next, steam explosion pretreatment of microalgae have also emerged as major interests, regarded as a promising method to enhance the efficiency of lipid extraction from microalgae. Steam explosion is performed by heating up the biomass by introducing steam to a chamber while gradually increasing the pressure to a certain level, and later releasing the pressure to atmospheric pressure in a rapid manner. The heating temperature is usually set as 160 °C or higher, while the pressure can go as high as 3.45 MPa [17,35]. Steam explosion are also sometimes known as thermal hydrolysis, the rapid drop of pressure causes cell wall to rupture, and therefore achieving cell disruption. Catalyst could also be added to increase pretreatment efficiency, e.g. sulphuric acid and sodium hydroxide [35]. Studies has

also shown that steam explosion could enhance the lipid extraction efficiency [19,35] or increase biogas production [38]. An overview of steam explosion pretreatment on different microalgae species is given in Table 4. Based on the study conducted by E.Lorente et al. [35], pretreatments show little improvement on lipid recovery for microalgae species like *Nannochloropsis gaditana*, *Chlorella sorokiniana*, *Phaeodactylum tricornutum* as compared to the fresh unprocessed sample. Among the pretreatment methods, it has to be stressed that steam explosion pretreatment gave the highest lipid extraction yields for these three microalgae species. Using higher temperatures both for the steam explosion treatment and extraction process can promote the lipid recovery efficiency [35].

Table 4 Summary of steam explosion pretreatment on different microalgae species

Microalgae species	Condition	Methane productivity (mL CH_4 g VS^{-1})	Lipid recovery/ efficiency/outcome	References
Nannochloropsis. gaditana	100 g of microalgae sample, 120 °C/150 °C for 5 min	-	8.1% / 8.4% wt. increment in lipid recovery	[35]
Chlorella sorokiniana	100 g of microalgae sample, 120 °C for 5 min	-	7.2% wt. increment in lipid recovery	
Phaeodactylum tricornutum	100 g of microalgae sample, 120 °C for 5 min	-	2% wt. increment in lipid recovery	
Chlorella vulgaris	160°C, 20min	0.156	65% wt. increment in methane yield	[17]

3. Non-mechanical Pretreatment

Non-mechanical pretreatments are the gentler approach to achieve cell disruption. Chemicals or enzymes can be chosen specifically to target the cell walls. Generally, non-mechanical pretreatment is a great option for mild disruption of cell wall, which prevents the degradation of the intracellular compound without going through extreme physical condition such as high shear stress and high temperature. However, the drawbacks of this method are requiring longer processing time and limited variety of enzyme or chemical available for cell disruption [21]. Since the efficiency of the same enzyme or chemical varies with the different type of microalgae species as enzyme or chemical works by selectively disrupting the specific cell wall component. For instance, to target the protein extraction from microalgae, cellulases and lipases were used as the specific enzymes to degrade the cellulose and phospholipids respectively. Therefore, the effectiveness of non-mechanical pretreatment is greatly dependant on the type of enzyme or chemical used during the cell disruption process, due to the highly specificity of enzymatic or chemical mechanism [14].

There are few aspects that must be considered before choosing the type of chemical or enzyme used, which are the type microalgae species, duration to achieve cell disruption, cell content to be extracted, and the cost. These aspects will be discussed in the following sections.

3.1 *Enzymatic Cell Disruption*

Enzymatic pretreatment is gaining much attention due to their ability to target specifically parts of the cells, which make it possible to mildly disrupt the cell walls. As compared to mechanical pretreatment, enzymatic approach is less energy intensive and less aggressive. The mechanical pretreatment methods are achieved through applying high stress forces on the cell wall whereas enzymatic pretreatment can be used to target a specific chemical bonding of the cell wall and therefore does not affect the intracellular contents [12, 14, 15, 21, 22]. Generally, enzymes are used to target the extraction of lipids from the microalgae [12, 26]. Multiple enzymes have been reported by researchers

to be effective on certain microalgae species [19–21,40]. For instance, the enzymes cellulase, xylanase, and pectinase has been reported to work on *Chlorella sp*. at the temperature of 55 °C and pH value of 4.8 [21]. Increase in lipid yield has also been recorded when the enzymes are mixed during the pretreatment process [41]. Mixture of cellulase, xylanase, and pectinase has also shown an improve in cell disruption on *Scenedesmus sp*. microalgae species at 45 °C with pH value of 4.4 [40]. For *Nannochloropsis* microalgae species, cellulase and mannanase has also reported to be successful at cell disruption [42], where the cellulase is naturally used to catalyse the hydrolysis of cellulose, which is the major component that sustaining the structure rigidity of microalgae cell wall [14]. Furthermore, increase in effectiveness of enzymatic pretreatment has been recorded by mixing cellulose and mannanase enzymes, where the cellulase and mannanase synergistically improved the recovery of lipids from *Nannochloropsis*. The yield of lipid extraction from the enzymatically treated *Nannochloropsis* sample increase 32.2% as compared to untreated *Nannochloropsis* sample [42].

Mixture of cellulase and lysozyme are also recorded to be effective against *Scenedesmus sp*. and *Nannochloropsis* sp. [30,31]. Amylase has also been recorded to be effective against *Chlorella vulgaris* and *Chlorella sp.* species [32,33]. The down side to enzymatic cell disruption is that incapability of high cost enzymes to be used on multiple type of microalgae species and enzymes are unable to be reused after production, causing this method is not economically feasible [46]. An overview of the type of enzymes used against different microalgae species in enzymatic pretreatment is provided in Table 5.

Table 5 Types of enzymes used in pretreatment

Microalgae Species	Enzymes	Lipid recovery value	Reference
Chlorella sp.	Cellulase, xylanase, pectinase	123.3 mg/g	[41]
	Amylase	137 mg/g	[45]
	Cellulase	127 mg/g	
Scenedesmus sp.	Cellulase, xylanase, pectinase	138 mg/g	[40]
	Cellulase	79 mg/g	[31]
	Lysozyme	82.2 mg/g	
Nannochloropsis	Cellulase, mannanase	388.4±0.95 mg/g	[42]
	Cellulase	85.33 mg/g	[43]
	Lysozyme	81.09 mg/g	
Chlorella vulgaris	*F. yaeyamensis* enzyme	218.6 mg/g	[44]

3.2 *Chemicals*

Pretreatment can also be performed using chemicals besides than enzymes. The most common chemicals are acids (e.g. sulphuric acid H_2SO_4, hydrochloric acid HCl, formic acid HCOOH etc.) and alkalis (e.g. sodium hydroxide NaOH, Potassium hydroxide KOH, Sodium Carbonate Na_2CO_3 etc.). Alkali addition causes swelling of lignocelluloses which are resistant to hydrolysis due to their structure and composition [18]. Hence, alkali reagents helps solubilize polymers, favouring the availability of organic compounds for enzymatic attacks [17,18]. On the other hand, acid pretreatment, often combination with heat, work by breaking down hemicellulose and disrupting ether bonds between lignin and hemicellulose [18].

Acid and alkali can be used to extract various type of content from the microalgae depending on the species. Sulphuric acid can be added to *Chlorococcum sp.* microalgae culture at 120 °C – 160 °C with 3 vol% – 8 vol% concentration to encourage cell disruption [29]. Report has also shown that HCl and formic acid can be used for cell disruption of *Chlorella prototheco*ides, *Nannochlorum*, and *Nannochloropsis ocenica* for lipid extraction [47]. Increase in effectiveness has also been recorded

when both acids are used together [47]. Sulphuric acid, H_2SO_4, can also be used to perform cell disruption on microalgae species *Chlorella vulgaris* for lipid extraction [41]. Other than lipids, acid and alkali can also be used to extract carbohydrates and/or protein [48]. Experiment has been performed that shows lime or calcium oxide, CaO, can be used for cell disruption on *Chlorella sp*. and *Scenedesmus sp*. to extract carbohydrates and protein [48]. Acid like urea, NaOH, has also shows similar result at cell disruption on *Chlorella vulgaris* for carbohydrates and protein extraction [49]. As for glucose extraction, cell disruption has proven to be effective using acid (sulphuric acid, H_2SO_4) or alkali (sodium hydroxide, NaOH) together with enzyme (cellulase + β-glucosidase) [50]. Other than acid and alkali, chemicals like solvent (e.g. acetone), antibiotics (e.g. penicillin), detergent, and hypochlorite (e.g. NaClO) have also been proven to be successful in achieving cell disruption [32,51]. Nickel oxide, NiO has shown positive result at cell disruption for *Chlorella vulgaris* [52]. A combination of triethylamine and methanol is reported to be effective at simultaneous cell disruption and extraction of lipids content from microalgae [53]. An overview of different types of chemical used in pretreatment of different microalgae species are presented in Table 6. Compare with enzymatic disruption, the cost to perform other chemical pretreatment can be much lower, especially the most common acid and alkali. Chemicals like solvents can also be used together with mechanical methods to further enhance the cell disruption effectiveness, as most of the chemicals are not effective enough to cause cell disruption alone [23].

Table 6 Types of chemicals used in pretreatment

Microalgae Species	Chemicals	Yield Extracted (%) = (weight of total lipid/ weight of biomass) x 100	Reference
Chlorella vulgaris	Nickel oxide, NiO	91.08	[52]
	NaOH	-	[49]
Chlorella sp.	Triethylamine + methanol 3:7	92.5	[53]
Chlorella protothecoides, Nannochlorum, Nannochloropsis ocenica	HCl	47.2 ±2.6	[47]
	Formic acid	46.7 ±0.8	
	HCL+formic acid	45.6 ±0.8	
Chlorella sp., Scenedesmus sp.	10% Lime, CaO at 72°C	77.9±0.6	[48]
Microalgae mixture	H_2SO_4/NaOH + (cellulase + β-glucosidase)	-	[50]
Chlorococcum sp.	Suphuric acid, H_2SO_4	-	[29]

4. Comparison of different cell disruption methods

The scalability and energy balance of microalgae pretreatment technique are the major concerns for the researchers, large amount of fuel must be produced each day to supply the demands if biofuels are to be used as a substitution for traditional fossil fuel. Although some methods are not suitable for large amount of biofuel production, the costing and simplicity of the pretreatment process may benefit the researchers as the samples of pre-treated microalgae may be needed to perform certain experiment. Numerous microalgae pretreatment are discussed above and each method has been proved to be efficient in microalgae cell wall rupture, improving the yield of lipid and carbohydrates extraction. Yet, an energy efficient and economical way of pretreatment for large industrial-scale microalgae biofuel production is still under research. For a profitable approach, we need to understand the strength and limitation of each pretreatment method, as the wrong choice of pretreatment can make the entire process uneconomical. The strength and limitation of these various existing pretreatment methods are

evaluated according to few important aspects, including the feasibility in large quantity fuel production, energy consumption, duration of pretreatment, cost and sustainability. A tabulation of review of each microalgae pretreatment mentioned in this study is shown in Table 7.

Table 7 Review of each microalgae pretreatment

Pretreatment method	Scale	Energy consumption	Duration (laboratory-scale)	Sustainability/Limitation	References
Bead milling	Laboratory-scale/ industrial-scale application	High/Medium	30~90min	High energy demand. Energy transfer to the individual cells from the rotating shaft is inefficient. Energy is lost in form of heat. Thus, extra energy is required for cooling process to allow the recovery of functional fragile products.	[19–22]
High pressure homogeniser	Laboratory-scale /industrial-scale application	High/Medium	5~30min	Induce degradation of compounds caused by the intense interfacial shear stresses and inherent heating occurring in the homogenization valve	[19,21,26–28]
Ultrasonication	Laboratory-scale	Medium/Low	4~15min	Improved substrate solubility but reduce efficiency of specific biogas production. Intense cell disruption can alter the chemical composition of the culture media	[17,30,33,34]
Microwave	Laboratory-scale/ industrial-scale	High/Medium	5~30min	Most of the radiation energy is exposed to the surrounding medium causing heat lost, protein aggregation and denaturation	[17,19,21]
Steam explosion	Laboratory-scale/ industrial-scale application	High	5~20min	Efficient and economical pretreatment method for fractionating and modifying lignocellulosic materials to improve the biomass feedstock quality for downstream processing	[17,19,25,35]
Chemicals	Laboratory-scale	Medium/Low	30min~120 hours	May form undesirable side products in the end product together with the reagents. Specific downstream treatment process is required.	[19,21,32,33,44]
Enzyme	Laboratory-scale	Low	30min~6 days	Not economically feasible, low production efficiency compared to other pretreatment methods. Required long reaction time and product inhibition.	[18,20,44]

For mechanical methods, bead milling, high-pressure homogeniser, and steam explosion at certain extends are suitable candidates for large amount of biomass pretreatment. On the other hand, microwave and ultrasound pretreatment are simpler to operate but could not compete in term of quantity. Ultrasonication pretreatment could also be configured to perform mild cell disruption, which will further increase the quality of biomass for fuel production.

Energy consumption, duration, and cost would also affect the feasibility in actual production. Theoretically some methods can be used for large amount of microalgae biofuel production, but other aspects must be put into consideration if the methods are to be implemented for real world applications. For instance, the scaling up of bead milling and ultrasonic pretreatment technique for industrial-scale production is still not feasible, as high energy input is required for large scale cell disruption, which result in no net or negative energy balances throughout the production of biofuels [17,19]. Besides, the duration of the pretreatment and the energy consumed would affect the cost, longer process or higher energy consumption would significantly increase the costing needed to sustain the production. Mechanical methods are more energy demanding, while the non-mechanical methods usually time consuming. Nonetheless, mechanical pretreatments can be improved by introducing chemicals or enzymes during the pretreatment process. Combination of both methods may significantly increase the efficiency while preserving the benefits from both methods.

On the other hand, enzymatic pretreatment serves a distinguish advantage which is mild disruption and highly specificity of enzymatic disruption mechanism. This avoid serious damage to the intracellular compound under the absence of toxic chemicals [20]. Despite the advantage, enzymatic cell wall degradation is not widely practiced in industry currently [20]. The high cost of enzymes stems generally cannot be recovered and recycled after production. Besides that, another drawback of enzymatic pretreatment is the limited availability and variety of suitable enzymes for microalgae disruption, due to their high substrate selectivity [18,20,44]. Hence, enzymatic pretreatment is not widely practiced in large scale industrial production [20].

For sustainability aspect, chemical pretreatment implemented at large production may cause more sustainability concern as compared to other pretreatment method. As shown in table 7, the addition of chemical reagent into the biomass solution may cause the formation of undesirable product and affect the downstream process. For instance, during continuous fermentation, alkali pretreatment leads to salt build up and increased pH of the microalgae biomass solution. The high salt concentration and the resulting effect on the ammonium-ammonia balance inhibits the fermentation process to produce biofuel [18]. Besides, this pretreatment technology is economically unattractive due to the high costs of alkalis and requires to carry out additional downstream process treatment [18,19]. Meanwhile, mechanical pretreament method shows lighter impact on sustainability and environmentally, due to the cell disruption mechanism is non-corrosive and has relatively lower maintenance cost during pretreatment.

5. Conclusion

Pretreatment process is crucial in aiding the extraction of microalgae content and helps improving biofuel production. With pretreatment the efficiency of fuel production can be greatly improved. There are multiple types of pretreatment methods available, each having its own advantages and disadvantages.

Mechanical methods are widely used due to their simplicity to operate and perform, it can also be used on multiple type of microalgae species. More importantly, the ease of scaling-up cell disruption processes to sufficiently large scale for biofuel production makes mechanical pretreatment a more preferable option. In this respect, bead-milling and high pressure homogenizer are considered as the most feasible method for industrial-scale application due to its low potential of contamination, cost effectiveness and higher net of energy produced [21]. On the contrary, ultrasonication pretreatment is an effective cell disruption method, but requires high energy consumption results in having negative

energy balances throughout the biofuel production. Microwave pretreatment simply relies on the disruption of hydrogen bonds that produces modifications in lipid structures [19]. Microwave pretreatment method are simple, better scalability, and efficient method for lipid extraction from microalgae [47]. Steam explosion pretreatment is a reliable method for effective cell disruption and enhancing organic matter solubilisation [17,35]. This method is under investigation at laboratory scale and shows great possibility for implementation in large industrial-scale [35]. On the other hand, enzymatic and chemical pretreatment can be used for mild cell disruption while at the same time greatly reduce the energy required to perform cell disruption. Despite the high cost of non-mechanical pretreatment method, chemical and enzymatic pretreatment show remarkable result in lipid/sugar recovery when combined with other mechanical pretreatment [35].

Therefore, it is important to study and understand the performances of these methods before a decision can be made at choosing the best methods. As technology improves and researches are progressively on-going, the most effective methods today may potentially be replaced in the future.

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