Microalgae Oil Extraction Pre-treatment Methods: Critical Review and Comparative Analysis

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Abstract

Microalgae biomass can be used to produce numerous value added products such as biodiesel, bioethanol, biogas, bioglycerol, fish feed, animal feed, human food supplements and skin care products. Production of value added products from microalgae biomass requires the growth and recovery of the algae biomass, extraction and downstream processing of the desired product. One of the major obstacles for using microalgae biomass on an industrial-scale, for the production of biodiesel, is the high processing costs. Increasing the lipid recovery efficiency from the microalgae biomass would result in greater product yields (biodiesel). Thus, the aim of this study was to review the current methods used for microalgae pre-treatment and perform a comparative analysis in order to determine the most economically efficient method for large scale use. The effectiveness of the pre-treatment methods investigated was evaluated based on: (a) cell wall disruption efficiency, (b) cost, (c) toxicity (d) suitability for large scale use, (e) time, (f) reusability and (g) maintenance. Different treatment methods included mechanical techniques (shaking vessel and agitated bead mills and horn and bath sonication), thermal methods (steam explosion, freeze drying and autoclave), electromagnetic radiation (microwave) and biological treatments (enzymatic). The results indicated that of the 9 microalgae methods investigated a mechanical, thermal and electromagnetic radiation techniques were suitable. These methods were bath sonication (81), steam explosion (93) and microwave radiation (87). Microwave assisted microalgae pre-treatment technique is rapid, effective in cell wall disruption, non-toxic, can be used for large volumes and the medium maybe reused, but it does however suffer from high maintenance costs. Bath sonication technique is effective in the degradation of cell wall, nontoxic, rapid technique with minimal maintenance required, but suffers from high costs and difficulty in scale up for industrial use. Steam explosion pre-treatment is effective in degrading microalgae cell wall, releasing intracellular components, rapid, reusable, relatively low in costs, environmentally friendly and reusable, but is species specific. Overall, the negative aspects of these three techniques are outweighed by their effectiveness, rapidness and relatively low costs when compared to other pre-treatment techniques. Other mechanical extraction methods suffer from high operational costs, lengthy treatment times, high maintenance costs and the scale up difficulty. Freeze drying and autoclave techniques were deemed unsuitable microalgae pre-treatment techniques because of the high costs, scale up difficulty and long processing times associated. Biological pre-treatment technique were deemed unsuitable as a result of high costs associated with purchasing of enzymes, difficulty in recovery/separation after treatment, long treatment time, and high maintenance required for high efficiency.

Keywords: Microalgae; Oil extraction; Lipid

Introduction

One of the most important resources for humans today is energy and its sustainability [1]. Today, petroleum products contributes 80% of the world's energy need as a fuel source for transportation and other energy demanding sectors [2,3]. Millions of years were required for the formation of fossil fuels and the diminishing crude oil reserves, increasing fuel prices and environmental concerns associated with fossil fuels usage make them unsustainable energy sources [4-6]. Renewable biofuels are regarded as an important energy resource in many countries globally, but only account for 10% of the total energy consumption [6].

There are three main generations of liquid biofuels [7]. The first generation of liquid biofuels includes both bioethanol and biodiesel production from food crops (corn, vegetable oils and sugarcane). This first generation of liquid fuels presents a problem with food supply and increased cost of food crops, thus limiting their use as energy sources. The second generation of liquid biofuels uses waste cooking oils, animal fats and non-edible plant seeds [7,8]. This second generation of biofuels solve the problem associated with the first generation of biofuels as these sources are not edible, but maintaining a consistent feedstock is a challenge. This inconsistency with feed stock supply gave rise to a third generation of liquid biofuels from aquatic algae [9].

Biodiesel from algae is a green alternative that reduces CO, CO₂ and hydrocarbon emissions compared to the currently used diesel fuel [10,11]. However, the problem associated with the use of algae for biodiesel production is the high costs associate with the algae production and oil extraction processes. In order for microalgae biodiesel to be economically viable, the processing needs to be made more economic by improving the methods of algae production, harvesting and oil extraction. This can be done by optimizing the algae production and harvesting processes and the oil extraction process for use on large scale. In particular, reducing the costs associated with lipid recovery and maximizing the recovery of the lipids in the biomass is vital for economically viable biodiesel production process, since these lipids are transesterified into biodiesel [12].

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The objectives of this study were (a) to review the current methods used for pre-treatment of microalgae for enhancing oil extraction (b) to review the current methods used for oil extraction from microalgae and (c) perform a comparative analyses in order to determine the most economically viable and efficient algae pre-treatment and oil extraction methods from microalgae at a large scale.

**Microalgae Lipids**

Microalgae biomass is composed of proteins, carbohydrates, lipids and nucleic acid that vary widely in proportion (Table 1) depending on the species cellular response [13-15]. There are numerous applications in which these components can be used such as health food additives for human consumption, animal feed, plant fertilizers and/or biofuels [4,13].

Lipids are contained as small spherical droplets in the chloroplast and between the thylakoid membranes as shown in Figure 1 [16]. They function in the structural support for the cell, the metabolic organelles in photosynthesis metabolism, the growth process of the cell and in the synthesis of lipoprotein membranes contained in the chloroplast [17]. Biodiesel production is generated through the conversion of lipids into fatty acids methyl ester using transesterification process as shown in Figure 2 [18-20]. Thus, lipids must be extracted from the microalgae biomass in order to avoid product contamination from other cellular

<table>
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<tr>
<th>Species of sample</th>
<th>Proteins</th>
<th>Carbohydrates</th>
<th>Lipids</th>
<th>Nucleic acid</th>
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**Table 1**: Microalgae composition [15].

![Figure 1: Schematic depicting the location of lipids microalgae cells and the process of formation [16].](image-url)
components.

Mechanical Pre-Treatment of Microalgae

Mechanical pre-treatment of algae to disrupt the cell wall and enhance the efficiency of the lipid extraction process by enhancing the solvent/lipid contact. Disruption of the cellular wall allows for easier recovery of the intracellular lipids resulting in rapid and increased efficiencies in lipid extraction [10,21-23]. Mechanical pre-treatment methods include bead milling and ultrasound.

Bead milling

Bead milling is a process that works to disturb the extracellular wall of microalgae by grinding and agitation of the cells on a solid surface of glass beads [24]. Exciting the beads using a bead mill produces a high shear force that can destroy the microalgal cell walls [25]. The optimal diameter size of the beads for effective microalgal cell wall disruption is 0.3-0.5 mm [26,27]. These beads can be made of zirconia-silica, zirconium oxide or titanium carbide [27]. Figure 3 and 4 depicts the difference between a disrupted microalgae cell with one that has not been pre-treated [23,26].

Types of bead milling

Figure 2: Transesterification of algal lipid into biodiesel [20].

Figure 3:Chlorella microalgae species before and after treatment [26].

Figure 4:Nannochloropsis sp. cells [23].
There are two types of bead milling vessels: shaking vessels and agitated vessels.

**Shaking vessels:** The shaking vessel works to disrupt the cell walls through the shaking of the entire culture vessel [28]. The vessel or multiple vessels are placed on a platform that vibrates allowing the beads to move and collide with the cells. The use of this type of bead mill is limited to laboratory scale and is not as effective in damaging the cell walls as agitated beads method [28,29]. Figure 5 shows a schematic of shaking vessel [30].

Zheng et al. [29] used a bead milling vessel to extract lipids from *Chlorella vulgaris* and noted a recovery of 11%, which was lower than other methods tested. Shen et al. [31] noted that the highest lipid recovery content of 18.8% from *C. protothecoides* was achieved using bead beater shaking vessel. Lee et al. [10] noted that bead beating of *Botryococcus* sp. cells resulted in a lipid extraction of 28%. Prabakaran and Ravindran [32] reported a lipid content recovery of 25-30% from *Chlorella* sp., *Nostoc* sp. and *Tolyphothrix* sp. species using bead beating. Ryckebosch et al. [33] noted a lipid recovery efficiency of 40% from *P. tricornutum* using a shaking beat beater.

**Agitated beads:** In this method, the beads and the culture are both agitated. A rotating agitator inside the vessel supplies the heat. Agitating the beads provides better disruption of the cell walls which increases the extraction efficiency. Figure 6 illustrates a schematic of the bead milling system [34]. For heat-sensitive molecules, the vessel is equipped with cooling jackets. This technique provides agitation, collision and grinding of the biomass which results in effective disruption efficiencies [35].

Gouveia et al. [36] extracted 33 g of oil from 100 g of *Nannochloropsis* sp. using bead mill assisted techniques. Halim et al. [37] found that agitated bead beating resulted in the disruption of 17.5% of *Chlorococcum* sp. microalga. Baldev et al. [38] reported a 2 fold increase in lipid yield in *Scenedesmus* sp. using agitated bead beater and mechanical grinding of the cells. Lee et al. [10] used bead milling and reported an oil yield range from 7.9-8.1 g/L from *Botryococcus* sp., *Chlorella vulgaris* and *Scenedesmus* sp. Shen et al. [31] noted an oil recovery of 20.5% using bead milling pre-treatment from *Chlorella protothecoides*. Ceron et al. [39] found that bead milling was the best method for lipid recovery from *Scenedesmus almeriensis*.

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**Figure 5:** Schematic of bead shaking vessel, jar is held in place inside the vessel and agitated [30].

**Figure 6:** Agitator bead mill unit [34].
Factors affecting bead milling

The degree of disruption of cells is depended on the strength of the microalgae cell walls, contact between the cells and the beads, as well as the shape, size and composition of the beads [26]. However, the biomass concentration, residence time and agitator speed have been identified to be most influential on the cell wall degradation efficiency, processing time and on the energy consumption [22,40].

Biomass concentration: The biomass concentration is one of the primary factors affecting the efficiency of microalgae cell wall disruption [40]. Increasing the biomass concentration in a pre-treatment bead mill leads to a higher fraction of microalgae cell disintegration as shown in Figure 7 [40]. The authors found that increasing the biomass concentration in bead milling of Chlorella vulgaris over the range of 25-145 g/L resulted in higher disintegration efficiency.

Doucha and Livansky [26] reported that increasing the Chlorella biomass concentration in bead milling resulted in increased disintegration of microalgae cell wall. Mogren et al. [41] also noted that disintegration rates increased with increasing concentration of yeast biomass in bead milling pre-treatment. Greenwell et al. [22] recommended that the cell concentrations should range between 100 to 200 g/L for high efficiencies and suitable economics, energy-wise.

Agitator speed: The agitator speed is another primary determinant of the efficiency of microalgae cell wall disruption. Postma et al. [40] stated that increasing the speed of agitation increases the impact force and the frequency which results in higher breakdown of cell wall, to a certain degree as shown in Figure 7 [40]. The authors found that increasing the agitator speed of bead milling in Chlorella vulgaris culture over the tested range of 9 - 12 m/s resulted in higher disintegration efficiency, but also noted an optimum speed in the range of 9-10 m/s. Doucha and Livansky [26] noted that increasing the agitator speed in bead milling processing of microalgae resulted in increased cell wall destruction. Hedenskog et al. [42] reported cell destruction efficiency in Scenedesmus quadricauda biomass of 55% using a speed of 2800 rpm. Lee et al. [10] noted lipid recovery efficiencies of 7.9-8.1 g/L from Botryococcus sp., Chlorella vulgaris and Scenedesmus sp. using a rotational speed of 2800 rpm. Bert et al. [43] noted that increasing the agitator speed from 8 to 14 m/s increased the dispersion efficiency of the agglomerate.

Residence time: The residence time is another primary determinant of the efficiency of microalgae cell wall disruption [22]. The fraction of microalgae cell disintegration as a function of time is depicted in Figure 8 [40]. Increasing the time of bead mill processing leads to higher cell disintegration until maximum disintegration is reached.

Doucha and Livansky [26] found that the breakdown of microalgae cell wall using bead milling increased from 67% to 95% as the treatment time increased from 30 min to 90 min, respectively. Postma et al. [40] noted that 500s of bead mill pre-treatment of Chlorella vulgaris biomass resulted in 99% disintegration, but 90-95% disruption was achieved at 200-250 s. Hedenskog et al. [42] reported cell destruction efficiency in Scenedesmus quadricauda biomass of 87% in 5 min. Safi et al. [44] reported that increasing the bead milling time increased the cell wall disintegration which lead to greater protein and pigment recovery efficacies from Chlorella vulgaris, up to the maximum reached at 40 min.

Bead density: The bead filling volume plays a role in the effectiveness of cell wall disruption. Increasing the contact between the particles and the cells by increasing the bead density enhances the dispersion up to a limit of 85% [43]. Doucha and Livansky [26] reported that the cell wall destruction values of 65, 83 and 85% were achieved from Chlorella using chamber bead volumes of 60, 75 and 80%, respectively. Hedenskog et al. [42] noted that 33 and 50% bead filling of the vessel resulted in a 55% and 90% cell disintegration of Scenedesmus quadricauda biomass, respectively. Liang et al. [45] extracted 38% of lipids from Chlorella vulgaris using 71% bead filling volume. Schweinzeier et al. [46] used a bead filling volume of 65% for the algae Tetraselmis sp. biomass for effective cell disruption for protein recovery. Bert et al. [43] noted that the dispersion efficiency was improved with increasing bead filling volumes over the tested range of 70-85%, but bead fill volumes greater than 85% significantly increased the wear of the mill.

Bead type and size: Varying beads and bead sizes have different impact on the dispersion efficiency [43]. Hedenskog et al. [42] noted a cell disintegration of 55% using ballotini beads (0.35-0.5 mm) in Scenedesmus quadricauda biomass. Doucha and Livansky [26] found
that zirconium dioxide beads (0.5 mm) and glass beads (0.42-0.58 mm) resulted in *Chlorella* biomass cell disintegration efficiencies of 98.5 and 99.9%, respectively. Lee et al. [10] noted lipid recovery efficiencies of 7.9-8.1 g/L from *Botryococcus*, *Chlorella vulgaris* and *Scenedesmus* sp. using glass bead (0.1 mm diameter).

Zheng et al. [29] used glass beads (0.4-0.6 mm diameter) to recover 10% of lipids in *Chlorella vulgaris* biomass. Lee et al. [47] reported a lipid recovery efficiency of 28.6% from *Botryococcus braunii* using 1 mm glass beads. Bert et al. [43] tested the effect of varying ceramic bead sizes (0.6-1.6 mm) on the agglomerate size and found that 0.8 mm beads were the most effective in achieving the lowest agglomerate size of 41 μm and ceramic beads significantly improved the dispersion efficiency compared to glass ones.

**Energy consumption**

Effective disruption of microalgae cell wall using bead mill pre-treatment has been noted to consume 35-810 Wh/kg of power [22,26,40]. The large variation in power consumption is a result of varying microalgae species and operation parameters.

Lee et al. [10] disrupted the microalgae *Botryococcus*, *Chlorella* and *Scenedesmus* using agitated bead mill with an energy consumption of 140 Wh/kg. Doucha and Livansky [26] noted that the power consumption for rupturing *Chlorella* using an agitated bead mill was in the range of 35-250 Wh/kg. Greenwell et al. [22] reported that the energy consumption for disruption of cell wall using a bead beater ranged from 300-400 Wh/kg. Postma et al. [40] noted an energy consumption of 810 Wh/kg for bead milling of *Chlorella* sp. biomass.

**Advantages and disadvantages**

The advantages of using bead beating are the simplicity, rapidness of the method, reproducibility of results and low labor intensity requirement [48,49]. However, pre-treatment of microalgae cells using bead milling can be difficult to scale up and requires the use of a cooling jacket in order to prevent the degradation of the desired product [10,25,48,50]. Additionally, bead mill pre-treatment is not a selective product recovery technique, which requires further processing to remove the undesired compounds [49]. The biomass undergoing bead disruption techniques must be dry and concentrated in order to achieve high disruption efficiency [49,51].

**Ultrasonication**

Ultrasonication is another mechanical method that can be used for pre-treatment of microalgae prior to lipid extraction. In this method, algae are exposed to high intensity ultrasonic waves, creating tiny cavitation bubbles around the cells. The bubbles collapse and emit shockwaves that shatter the cell walls causing the intracellular lipids to enter the bulk of the solution as shown in Figures 9 and 10 [24,52-54]. Ultrasonic assisted microalgae lipid extraction has been noted to significantly increase the yields and reduce the extraction time [10,24,55]. Pernet and Tremblay [56] concluded that the ultrasonic method for oil extraction from *Chaetoceros gracilis* increased the extraction rate which affects the recovery of lipid extracts. There are some contradictions in the literature regarding scale up. Halim et al. [37] noted that this technique is moderately suitable for scale up whereas Mercer and Armenta [24] stated that ultrasound maybe difficult for upscale.

Wiltshire et al. [57] reported a 90% extraction efficiency of fatty acids and pigments from the species *Scenedesmus obliquus* using ultrasound extraction. Ranjan et al. [58] reported that ultrasound assisted microalgae lipid extraction demonstrated more distorted clusters of biomass on micrographs, in comparison to cells with solvent penetration. Cravotto et al. [59] noted that ultrasound assisted lipid extraction from *Cryptothecodinium cohnii* resulted in an increase in lipid yield of 21.1% as opposed hexane solvent extraction.

Lee et al. [10] tested various cell disruption techniques (autoclave, bead milling, microwave and sonication) on lipid extraction from *Botryococcus* sp. and found that sonication was the least efficient in lipid extraction, but the yields were higher than that with sole solvent extraction. Shen et al. [31] reported that the lipid content for *Chlorella protothecoides* was least using sonication treatment compared to methods of bead beating and press. Zheng et al. [29] tested treatment of *Chlorella vulgaris* biomass using microwave, ultrasound, enzyme lysis and bead beating, which resulted in lipid yields of 18, 15, 22 and 10%, respectively. Prabakaran and Ravindran [32] found that the lipid recovery from *Chlorella* sp., *Nostoc* sp. and *Toxothrix* sp. was highest using sonication pre-treatment compared with auto-calving, bead beating and microwave. De Souza Silva [60] tested the pre-treatment of microalgae culture using microwave, autoclaving and ultrasonication.

![Figure 8: Microalgae fraction of cell disintegration as a function of time (40).](image-url)
Figure 9: Micrographs of microalgae before and after ultrasound treatment [53].

Figure 10: Schematic of cell disruption using ultrasound energy [54].

Figure 11: Horn sonication unit [65].
technology for lipid extraction and found that ultrasound resulted in the lowest yields. Koberg et al. [61] reported a lipid yield of 18.9% and 32.8% in Nannochloropsis species using microwave and ultrasonication pre-treatment, respectively.

**Ultrasound types**

There are two types of ultrasound units: horns and baths [62]. Both of these types are used for batch operations, but the addition of flow cells can alter them into continuous operational modes [63,64].

**Horn:** Horns type ultrasonic technology use a piezoelectric generator that is composed of lead zirconate titanate crystals that vibrate with amplitude ranging from 10 µm to 15 µm (Figure 11) [65]. The formed vibrations travel down the titanium metal horn or probe, increasing in amplitude ranging from 100 µm to 150 µm at the tip. The power at the tip should be of high intensity in order to create cavitation with sufficient disruptive force since the energy dissipates rapidly with distance. The use of horn type sonicators is limited to laboratory scale handling 10-100 mL volumes. Setting the horn to vibrate laterally would increase the area of contact for larger cavitation, but larger area would decrease the intensity of the cavitation. The scale-up of horns sonication would require the use of multiple systems and the use of continuous flow of the cells [35]. Jeon et al. [66] stated that disruption of microalgae biomass using horn sonicators is not suitable because the cavitation is localized. Wang et al. [67] noted that the relative lipid increase rate for S. dimorphus (30 mL) using horn sonication was lower than that using bath sonication at operational times of 1 and 5 min. Cravotto et al. [59] noted that horn sonication of the marine microalgae Cryptothecodinium cohnii (50 mL) was effective in increasing the lipid recovery. Menendez et al. [55] reported that horn ultrasonic treatment did not increase the lipid yields in Nannochloropsis gaditana microalgae, compared to conventional extraction techniques.

**Bath:** Ultrasonic baths use transducers, placed as the bottom of the reactor, to generate the ultrasonic waves (Figure 12). The number and the arrangement of the transducers vary with the capacity of the reactor and the shape. Sonicator baths have a larger capacity (up to 3 L), but the reactor size is limited by the rapid rate of sonic energy dissipation with distance. Baths can be modified in flow cells for continuous industrial operation and higher efficiencies may be achieved using multiple transducers operating with 2 or 3 varying frequencies in the cell. Wiyarno et al. [69] noted a lipid recovery of 26% from Nannochloropsis using ultrasonic bath treatment. McMillan et al. [70] investigated the disruption efficiency of Nannochloropsis oculata biomass using microwave, mechanical force and ultrasonic bath treatments and found that ultrasound was the least effective. Neto et al. [71] treated Chlorella minutissima, Thalassiosira fluviatillis and Thalassiosira pseudonana with ultrasonic bath waves and noted that ultrasonic was a necessary step for cell disruption in order to increase the lipid recoveries. Piasecka et al. [72] noted that sonication bath treatment of Chlorella protothecoides biomass resulted in an increase in lipid yields of 37%.

**Factors affecting ultrasonic assisted extraction**

The pre-treatment time, temperature of the reaction, cell concentration and microalgae type have been reported to influence the lipid yield in microalgae lipid extraction assisted with ultrasound technology.

**Extraction time:** Increasing the time in which the cells are exposed to ultrasound can affect the recovery rate. Longer treatment time allows for increased cell disruption as a result of additional energy input [35]. Menendez et al. [55] reported that an increase in extraction time from 5 to 20 min increased the lipid yield from 31 to 36%, respectively. Adam et al. [73] noted that increasing the treatment time resulted in higher lipid recovery efficiencies. Tang et al. [74] found that increasing the ultrasound treatment time over the range of 15 to 90 min has no significant effect on the microalgae lipid recovery. Wiyarno et al. [69] reported that an increase in sonication time resulted in higher lipid yields. McMillan et al. [70] found that increasing the sonication time resulted in greater cell disruption efficiencies. However, Prommuak et al. [75] found that increasing the reaction time from 15 to 30 min resulted in decreased lipid yields from Chlorella vulgaris and Haematococcus pluvialis species as a result of lipid oxidation with prolonged treatments.

**Reaction temperature:** Altering the reaction temperature during ultrasonic treatment has been noted to influence the lipid recoveries from microalgae biomass. Prommuak et al. [75] found that an increase in temperature from 30 to 40°C recovered slightly higher lipids after 5-10 min. Adam et al. [73] reported that increasing the temperature from 1 to 35°C in ultrasound assisted oil extraction from Nannochloropsis oculata resulted in increased oil yields by a factor of 1.5. Wiyarno et al. [69] noted that increasing the reaction temperature from 23 to 60°C resulted in increases in lipid yields.

**Cell concentration:** The effectiveness of microalgae cell disruption using sonication technique has been noted to change with cell concentrations. Lee et al. [35] stated that increased cell fragments build up during treatment reduces the efficiency of the process and concluded that sonication of concentrated biomass is less efficient in dilute suspensions. Nowotarski et al. [76] reported that sonication of the microalgae Dunaliella salina was most effective at low densities. Adam et al. [73] found that the optimal concentration of microalgae cells for lipid extraction using sonication was 5% over the tested range of 5-30%. However, Gerde et al. [77] found that varying the microalgae concentration over the tested range of 1.5-14.1 g cells/L did not require higher sonication energy for complete disruption. Natarajan et al. [78] reported that the cell disruption efficiency increased with an increase in cell concentration up to 6.84 g/L over the tested range of 0.07 g/L to 12.22 g/L.

**Microalgae type:** The components of the microalgae cell wall coating are species specific and play an important role in the disruption efficiency using sonication techniques. Diatoms have siliceous cell wall coating, called frustules and the degree of silification of the frustules varies with cells of the same species as a result of nutrient availability [79]. Neto et al. [71] reported that for lipid extraction from diatom microalgae, sonication greatly impacted the cell disruption and improved the oil recovery. Joyce et al. [80] reported that suspensions of Nannochloropsis oculata was unaffected by the ultrasound treatment as a result of varying microalgae cell wall thickness but Dunaliella salina and Chlorella concordia resulted in complete cell disruption with 4 min and 16 min sonication treatments, respectively. Nowotarski et al. [76] reported that sonication of Dunaliella salina was effectively disrupted at low concentrations while Nannochloropsis oculata was much more resistant to sonication even at long sonication exposures. Natarajan et al. [78] found that the Chlorella sp. cells released the lipids into the liquid suspension after sonication treatment but Tetraselmis suecica and Nannochloropsis sp. retained the lipids in the membrane after undergoing ultrasonication treatment.

Takeda [81] noted that lipid extraction from Chlorella minutissima did not improve with sonication treatment as a result of cell fragility compared to diatoms. Sostarcic et al. [82] reported an increased in lipid yield of 6.02% in Chlorella vulgaris biomass using ultrasonic bath treatment because the Chlorella species possess a thin cell wall made
up of sugar [83]. Kaivan-arporn et al. [84] compared lipid yields from Synechocystis aquatilis using grinding and sonication techniques and found that the yields were 21.3% and 10.2%, respectively. Shen et al. [31] noted that the lipid yield using sonication is affected by cell size, shape and structure and reported lipid yields of 10.7 and 21.2% for Chlorella protothecoides and Scenedesmus dimorphus, respectively.

**Ultrasonic power:** The ultrasonic power has been noted to have negligible effects on the oil yield of varying microalgae species. Natarajan et al. [78] tested ultrasonic power of 500, 750 and 1000 W on Tetraselmis suecica and Chlorella sp. oil yields and found that over the tested range no variations in yields were noted. Tang et al. [74] tested the effects of varying ultrasonic power on the lipid yield of microalgae over the range of 80-200 W and found no significant difference in the oil yields with varying power. Cravotto et al. [59], Singh and Gu [32] and Adam et al. [73] have also reported that the power of sonication has little effect on the oil yields of microalgae species.

**Energy consumption**

Menendez et al. [55] reported that the energy consumption using ultrasound assisted lipid extraction from Nannochloropsis gaditana increased from 1.7 to 6.7 Wh/g dried biomass when the reaction time was increased from 5 to 20 min which corresponded to increases in the lipid yields of 31 and 36%. Guldhe et al. [85] reported that the energy consumption using ultrasound assisted lipid extraction from Scenedesmus sp. was 119 Wh/g. Adam et al. [73] reported an energy utilization of 16.66 Wh/g for lipid extraction from the microalgae Nannochloropsis oculata. Halim et al. [37] noted that the sonication energy consumption for Chlorococcum sp. was 36.66 Wh/g of dried biomass, but the lipid yields were only slightly higher than that from untreated biomass. Bigelow et al. [86] found that sonication of Chlamydomonas reinhardtii microalgae biomass consumed 36 Wh/g. Wang et al. [67] noted that sonication of Scenedesmus dimorphus and Nannochloropsis oculata consumed energy in the range of 17.3-86.8 Wh/g.

**Advantages and disadvantages**

Pre-treating microalgae biomass using ultrasound has several advantages which include: reduced extraction time, less solvent requirement, high yields as a result of easy cell penetration, the biomass does need to be dry and easier release of intracellular components to the bulk of the solvent [87-90]. Chemat et al. [89] and Wang and Weller [90] stated that ultrasound assisted extraction can be operated at low temperatures (less thermal denaturation of biomolecules) and is much more economical compared to other conventional extraction methods. However, ultrasound assisted techniques consume large amounts of power and pose scale up difficulties [87,88]. The use of high-intensity sonication can result in pressurize/heat that is damaging to the cells or tissue [67]. This technique can be species specific since it has been noted to be ineffective in the destruction of diatom species with thick cellular coating [76,78,80].

**Thermal Pre-Treatment of Microalgae**

Thermal methods can be used to enhance the lipid recovery from microalgae by disrupting the cell wall which allows for the release of the intercellular lipids. Thermal pre-treatment methods of microalgae include steam explosion, autoclaving and freeze drying.

**Steam explosion**

Steam explosion may also be used to disturb the microalgal cell wall so that the intracellular components may easily be recovered. The treatment works by exposing the biomass to high temperatures that vary from 160°C to 260°C [91-93] and vapor pressure in the range of 1.03 and 3.45 MPa, followed by the return to ambient conditions through depressurization. The drop in pressure to ambient conditions results in cell wall rupture of the biomass [94-98]. Pre-treating microalgae biomass with steam technology should be performed at lower temperatures in order to prevent lipid degradation [95].

A batch unit setup for steam pre-treatment of microalgae is illustrated in Figure 13 [94]. The unit consists of a 4 L steam generator, 2 L reactor and a collection vessel. The sudden depressurization takes place in the collection tank which is equipped with a flash valve to allow the return to atmospheric conditions. This form of cell wall disruption is efficient and economically viable method [95].

Loren et al. [94] reported that steam explosion pre-treatment of three types of microalgae biomass resulted in the higher lipid extraction efficiencies compared to autoclaving, ultrasound and microwave techniques. Nurra et al. [95] noted that the steam explosion pre-treatment of Nannochloropsis gaditana biomass resulted in an increased lipid recovery from 0.3% to 3.6%. Mosier et al. [99] and Montane et al. [100] reported that steam explosion is an economically efficient pretreatment technique for fractionating and modifying lignocellulosic materials which improves the feedstock quality for downstream processing.

**Factor affecting steam explosion**

The factors which play an important role are steam explosion cell wall destruction of microalgae is temperature, pressure and microalgae species.

**Temperature:** The temperature plays an important role in the effectiveness of microalgae cell wall rupture [95]. The optimum temperature for hydrolysis of microalgae biomass depends on the microalgal species [101].

Loren et al. [94] found that steam explosion of Nannochloropsis gaditana at 120°C and 150°C resulted in an increase in lipid extraction yields of 8.1 and 8.4%, respectively. Nurra et al. [95] noted that increasing the temperature in steam explosion pre-treatment of Nannochloropsis gaditana biomass from 120 to 180°C increased the lipid yields from 3.6 to 8.8%. Du et al. [102] noted that pre-treatment of microalgae biomass using steam explosion at a temperature of 150°C for 50 min was not sufficient in destruction of the cell wall. Alzate et al. [101] reported that increasing the temperature from 110°C to 170°C resulted in increased microalgae biomass biodegradability (10% to 27%). Mendez et al. [103] reported an increase in protein solubilisation in Chlorella vulgaris biomass of 16% at temperature of 140°C and an increase in temperature...
in the range of 160 to 180°C resulted in solubilisation of 45%. Aguirre and Bassi [104] noted that an increase in temperature from 104°C to 210°C in *Chlorella vulgaris* increased the extraction efficiencies from 24 to 77%.

**Pressure:** Altering the temperature in the steam explosion treatment vessel, changes the pressure of the system which affects the solubilisation of the microalgae biomass. Aguirre and Bassi [104] stated that temperature and pressure are correlated in steam treatment. Lorente et al. [105] noted a slight increase in microalgae lipid recovery from 17.9 to 18.2% using steam explosion with increases in pressure from 2 to 4.7 bars which was achieved by increasing the temperature from 120 to 150°C. Nuraa et al. [95] reported an increase in lipid recovery from 3.6 to 8.8% using steam explosion pre-treatment of microalgae biomass as the pressure was increased from 2 to 10 bar by increasing the temperature from 120°C to 180°C. Mendez et al. [103] reported that with increasing pressure from 3 to 10 bars in steam explosion of *Chlorella vulgaris* biomass the carbohydrate solubilisation increased. Robles Medina et al. [105] noted that carotenoid extraction from *Haematococcus* biomass increased by 15% when treated with high pressure compared to no pretreatment techniques.

**Microalgae species:** The lipid recovery from microalgae species using steam explosion varies with the type of species as a result of different cellular composition. Lorente et al. [94] found that the lipid recovery using steam explosion resulting in lipid recovery increases of 8.7%, 9.5% and 2.1% compared to Bligh and Dyer extraction from *Nannochloropsis gaditana*, *Chlorella sorkiniana* and *Phaeodactylum tricornutum*, respectively. Alzate et al. [101] noted that steam explosion treatment for the production of biogas using *Chlamydomonas*, *Scenedesmus* and *Nannochloropsis* resulted in a biodegradability of 70% while *Acutodesmus obliquus*, *Oocystis*, *Phormidium* and *Nitzschia* sp. species resulted in a biodegradability of 30% using steam explosion treatment. Pandey et al. [106] stated that the effectiveness of steam pre-treatment of microalgae is dependent on the cell wall composition which varies among microalgae species.

**Cell concentration:** The biomass concentration has been noted to influence the effectiveness of the steam explosion technique. Aguirre and Bassi [104] found that the highest lipid yields from *Chlorella vulgaris* treated with steam, were achieved at concentrations lower than 5 g/L. Kita et al. [107] found that *Botryococcus braunii* treated with steam resulted in hydrocarbon recoveries of 90% or greater when operating at thermal temperatures of above 85°C with a biomass concentration of 1.5 g/L, while Frenz et al. [108] noted a recovery of less than 1% using cell concentrations of 1 g/L of *B. braunii*, thus concentrations above 1 g/L were recommended. Mendez et al. [55] reported that high pressure steam treatment at temperatures above 160°C of *Chlorella vulgaris* at concentrations of 16 g/L only solubilized 45% of the proteins, thus lower concentrations are required to achieve a higher degree of solubilisation.

**Energy consumption**

Very little work has been done on the investigation of steam explosion as a pretreatment technique on microalgae biomass. Keymar et al. [109] noted that the energy consumption for using steam explosion pre-treatment on microalgae biomass consumed 2.92 Wh/g of volatile solid. Ko et al. [110] reported an energy consumption of 1.11 Wh/g for steam explosion pre-treatment of Ma bamboo for the production of 1 L of ethanol. Zhu and Pan [111] found that the steam pre-treatment of wood biomass consumed 0.98 Wh/g.

**Advantages and disadvantages**

Steam explosion pre-treatment is advantageous as it disrupts the cell wall as a result of sudden pressure release, making the lipids accessible for rapid recovery [95] without the release of hazardous wastes [91,112]. Other advantages to steam explosion pre-treatment are its relatively low energy consumption [111,112], low maintenance costs and its low corrosion potential [112]. However, the effectiveness of this technique is species specific [106]. To date steam explosion pre-treatment of microalgae biomass has only been recently studied and is under investigation at laboratory scale and has been primarily used for the production of biogas product [106].

**Autoclaving**

Autoclaving (Figure 14) microalgae biomass is a form of thermal
treatment operating at a temperature of 121°C and pressure of 15 lbs [113,114]. High thermal stress causes the cell walls to rupture forcing the release of the intracellular lipids [32,37].

Surendhiran and Vijay [113] noted that autoclave pre-treatment of *Nannochloropsis oculata* biomass resulted in higher lipid yields than those without initial treatment. Lee et al. [10] also found that maximum oil recoveries from *Chlorella vulgaris* were achieved with autoclave pre-treatment. Prabakaran and Ravindran [32] reported that the lipid yield recovered from *Chlorella vulgaris* was higher with autoclave pre-treatment than that without pre-treatment, but the lipids were not higher than microwave treated biomass. De Souza Silva et al. [60] noted that autoclave and microwave pre-treatment of microalgal biomass resulted in lipid yields of 15.4 and 33.7%, respectively.

**Factors affecting autoclave assisted extraction**

The efficiency of autoclave technology is dependent on time elapsed of the treatment as well as the type of microalgae.

**Time:** Altering the autoclave treatment duration has been reported to effect the effectiveness of microalgal lipid recoveries. Surendhiran and Vijay [113] pretreated *Nannochloropsis oculata* biomass using autoclave operating at 121°C and a pressure of 15 lbs at 10, 20 and 30 min and achieved the highest lipid recovery of 29.34% at 30 min treatment. Prabakaran and Ravindran [32] noted an increase in lipid content of 22% in *Nannochloropsis oculata* with autoclave pre-treatment at 121°C for 5 min, but higher yields were achieved using microwave pre-treatment. Lee et al. [10] reported an increase in lipid recovery of 4% in *Botryococcus* when autoclaved for 5 min. Rakesh et al. [115] found that the autoclave pre-treatment of *Botryococcus* sp. for 15 min was effective in increasing the lipid recovery from the cells.

**Microalgae type:** The species type has been noted to effect the autoclaves ability to effectively disrupt the cell wall. Rakesh et al. [115] reported that autoclaving microalgal biomass for 15 min effectively increased the lipid recoveries in *Botryococcus*, but resulted in a 50% reduction in total lipid recovered from *Chlorella sorokiniana* species. Prabakaran and Ravindran [32] reported higher lipid recoveries in *Chlorella*, *Nostoc* sp. and *Tolypothrix* when autoclaved, however higher recoveries were achieved using microwave, sonication and bead beating since they are more abrasive destruction techniques. Lee et al. [10] found that autoclave pre-treatment was effective in increasing the lipid recovery in *Scenedesmus* sp., but was not effective on *Botryococcus* sp. and *Chlorella vulgaris* compared to other pre-treatment methods investigated. Yu et al. [116] reported that autoclaving *Chlorella sorokiniana* species resulted in the lowest lipid yields compared to other techniques such as bead beating, microalgae and sonication. Miranda et al. [117] noted that autoclave treatment of *Scenedesmus* biomass was the most efficient technique for sugar recovery. The effectiveness of autoclaving treatment on different microalgae species varies as a result of different cell wall structures that can be tough and unaffected by autoclave disruption techniques [117,118].

**Energy consumption**

The energy consumption for autoclave pre-treatment of microalgae biomass is high as a result of high operational temperatures and pressures [119]. This technique has been shown to consume higher amounts of energy than those of bead beating techniques [10]. Lopata et al. [120] reported that the costs of autoclave machinery range from high to very high. Panasonic [121] reported that their laboratory size (50 L) autoclave (Model No. MLS-3751L-PA) has a power consumption of 1900 watts. SciCan [122] noted a power consumption of 1700 watts (50 L) autoclave (Model No. MLS-3751L-PA) has a power consumption of 1900 watts. SciCan [122] noted a power consumption of 1700 watts for their Bravo laboratory scale (17 L) autoclave. Market Forge STM-E autoclave, higher capacity of 85 L has a power consumption of 9000 watts [123].

**Advantages and disadvantages**

The use of autoclave pre-treatment of microalgae biomass prior to lipid extraction is advantages because it disturbs the extracellular cell membrane allowing for easier recovery of lipids due to increased penetration [119]. Autoclaving cells at high temperatures over a short duration can reduce the degradation of the desired product [124]. However, this technique is difficult to upscale, long duration of time required, and large scale use would require high energy consumptions [119].

**Freeze drying**

Freeze drying (Figure 15) microalgae biomass is one of the most preferred drying techniques as a result of its mild operating conditions and the ease of lipid extraction after pre-treatment [50,125]. It illustrates the cell structure of microalgal species that have been freeze dried prior to lipid extraction [126]. The solvent extraction of lipids from wet biomass can be difficult, thus prior freeze drying of the biomass will overcome this difficulty [50]. However, microalgal lipids are susceptible to degradation under thermal drying techniques and can result in evaporative loss of lipids [127]. Further milling of the
freeze dried microalgae would enhance the efficiency of lipid extraction as a result of increased surface area for biomass-solvent contact and diminishes the diffusion pathway [37].

Guldhe et al. [85] stated that water needs to be removed from microalgae biomass in order to increase the lipid extraction efficiency. Pasquet et al. [126] found that freeze drying of the microalgae species Dunaliella tertiolecta preserved the integrity of the cell. Arora [128] noted that freeze dried microalgae biomass is more effective in the recovery of microalgae lipids as opposed to the solvent recovery using wet biomass. Belarbi et al. [129] reported that solvent extraction of oil from wet biomass can be difficult, but prior freeze drying of the biomass can extract lipids readily. However, Guldhe et al. [85] noted that solvent lipid extraction from Scenedesmus sp. dried using freeze-dryer; oven and sun drying techniques did not significantly differ from one another. Balasubramanian et al. [130] also noted that the drying type (freeze-drying, oven and sun drying) of Nannochloropsis sp. biomass has no significant influence on the lipid recovery using solvent extraction. Fujardo et al. [131] used freeze dried biomass in the solvent recovery of oil from P. tricornutum microalga species.

Energy consumption

The energy consumption reported in the literature for freeze-drying of microalgae biomass has only been investigated on a small laboratory scale. Guldhe et al. [85] noted that the energy consumption for freeze-drying Scenedesmus sp. biomass was 17.7 kWh for every gram lipid recovered. Green and Perry [132] reported that the energy consumptions for drying microalgae using a freeze dryer of 5.56 Wh/g. Bennion [133] found that the energy consumption for freeze drying microalgae biomass was 5.27 Wh/g. Ratti [134] calculated that the industrial freeze drying of biomass would require 38.88 Wh/g of biomass. Variation in the energy usage is a result of varying operational parameters and operational duration.

Advantages and disadvantage

Freeze drying of microalgae biomass breaks up the biomass cells and turns them into fine powder, eliminating the need for homogenization [135]. Freeze drying is one of the most commonly used technique for the production of high value products because it is gentle and does not have adverse effects on the cellular components [33]. Recovery of lipids from freeze-dried microalgae biomass does not require the use of a prior pre-treatment technique [129]. However, the process can be time consuming and the costs can be high [35,136]. Freeze drying has been deemed unsuitable for large scale operation as a result of high costs associated with scale up [50,136].

Electromagnetic Radiation

Microwave

Microwave pre-treatment processes enhance the kinetics of the lipid extraction process by disruption of the cellular wall [90,103]. This technique provides large amounts of thermal energy from electromagnetic radiation with a certain frequency to the cells [137,138]. A schematic of the microwave processing system is illustrated in Figure 17 [137]. The process consists of a feeding tank, microwave unit, water bath and a peristaltic pump that generates feed circulation through the system. Tubes with a diameter of 0.953 cm were used to carry the microalgae biomass from the feed-tank to the microwave unit. In the microwave chamber the mixture is heated using microwave radiation within seconds. Varying the radiation time alters the cell wall disruption efficiency. After treatment, the processed fluid is released into a 50 mL beaker that is held in a constant temperature water bath [137].

The thermal energy is a result of frictional forces that are caused by inter and intra-molecular movements [139]. This energy causes temperature and pressure effects on the cell wall that result in cell wall rupture [37]. Disruption of the cell walls allows for the cell components to be released which increases the efficiency of the extraction process by overcoming the concentration gradient associated with solvent extraction [10,140].

Balasubramanian et al. [137] investigated the use of microwave assisted solvent extraction for lipids from S. obliquus and noted that higher oil yields were achieved with solvent system hexane compared to the hexane extraction without microwave radiation at all temperatures and reaction times investigated.

Lee et al. [10] tested bead milling and microwave cell disruption techniques on lipid extraction from Botryococcus sp., Chlorella vulgaris and Scenedesmus sp. with chloroform/methanol (1/1 v/v) solvent system and found that microwave assisted method resulted in the highest the lipid yield for all three species. Sostaric et al. [82] reported that pre-treatment of microalgae biomass using microwave irradiation results in higher oil yields. Cheng et al. [140] noted that the use of microwave irradiation resulted in a 31% increase in oil yields. Sostaric et al. [82] postulated that pre-treatment of microalgae biomass resulted in higher bio-oil yields due to micro-cracks present in the cell wall.

Factors affecting microwave assisted extraction

Temperature, reaction time and energy input can impact the efficiency of microalgae lipid extraction using microwave pre-treatment technology.

Temperature: High amounts of thermal energy in the form of heat disrupt the cellular wall of microalgae organisms and allows for easier recovery of intracellular lipids [90,103]. Figure 18 depicts the effects of microwave pre-treatment on microalgae cells [141]. Increases in temperature result in rapid destruction of the cellular wall which results in increased lipid recoveries [75,103]. Figure 19 depicts the effect of microwave pre-treatment temperature on the lipid recoveries of varies microalga species [51].

Menendez et al. [103] noted that increasing the reaction temperature from 60 to 90°C in microwave assisted lipid extraction from Nannochloropsis gaditana resulted in increased extraction efficiency from 29 to 40%, respectively. Prommuak et al. [75] found that the lipid extracts from Chlorella vulgaris and Haematococcus pluvialis using microwave assisted techniques at high temperatures resulted in higher lipids. Balasubramanian et al. [137] reported that increasing the temperature from 80 to 95°C in microwave assisted lipid extraction resulted in higher lipid yields (23-30% dry weight) from Scenedesmus obliquus. Passos et al. [142] noted increased solubilisation of microalga biomass with increasing temperatures over the tested range of 50 to 98°C. Cheng et al. [140] reported an increase in microalga cell wall destruction with microwave pre-treatment as the temperatures increased from 80-120°C.

Time: The length of time in which cells are exposed to microwave radiation has significant influence on the effectiveness in the destruction of the cell wall, which determines the recovery efficiency of the lipids present in microalgae biomass [143]. Figure 20 depicts the relationship between varying the length of microwave treatment with the oil recovery [137].

Patil et al. [143] noted that the reaction time has a significant
Figure 16: Scanning electron microscope micrograph depiction of freeze-dried microalgae biomass [126].

Figure 17: Microwave processing system [137].

Figure 18: Microwave treatment of microalgae biomass [141].
effect on the effectiveness of the microwave assisted pre-treatment and that increased duration of microwave exposure increased the oil yields. Menendez et al. [103] found that increasing the reaction time from 10 min to 20 min in microwave assisted lipid extraction from *Nannochloropsis gaditana* at a temperature of 60°C resulted in increased extraction efficiency from 29 to 40%. Balasubramanian et al. [137] reported that increasing the extraction time from 10 to 20 min in microwave assisted lipid extraction resulted in an increased lipid yield from 10% to 22% for *Scenedesmus obliquus*. Dai et al. [144] noted that increased microwave extraction time from 10 to 40 min resulted in increased microalgae lipid recovery 14 to 18%.

**Applied energy:** The amount of microwave energy applied has been noted to effect the microalgae solubilisation. Dai et al. [144] noted an increase in microalgae lipid recovery with increasing microwave treatment power from 400 to 1000 W. Qv et al. [145] reported an increase in microalgae extraction efficiency with increases in microwave power from 140 to 560 W. However, the authors noted that further increase to 700 W decreased the extraction recovery. Biller et al. [51] found that increasing the microwave power from 25 to 61 Wh/g resulted in increased lipid yield from *Nannochloropsis* biomass from 1.6 to 10%. Passos et al. [142] noted that increasing the microwave energy from 300 to 900 W resulted in increased microalgae biomass solubilisation.

**Energy consumption**

The energy consumption of microwave pre-treatment depends on the temperature and duration of the treatment. Menendez et al. [103] reported that the energy consumption using microwave assisted lipid extraction from *Nannochloropsis gaditana* increased from 0.9 Wh/g to 1.6 Wh/g of dried biomass, as the reaction temperature increased from 60°C to 90°C which corresponded to lipid yields 29% and 40%. Balasubramanian et al. [137] extracted 76-77% of the oil from *Scenedesmus obliquus* using microwave radiation with an energy consumption of 60 Wh/g of dried biomass. Guldhe et al. [85] found that the energy consumption for microalgae lipid extraction from *Scenedesmus* sp. was 98 Wh/g of dried biomass at a temperature of 

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**Figure 19:** Effect of increasing microwave treatment temperature on the lipid extraction of microalgae [51].

**Figure 20:** Varying microwave treatment time on *Scenedesmus obliquus* biomass with oil recovery efficiencies [137].
100°C. Bermudez et al. [146] noted that the energy consumption for microwave assisted lipid extraction from microalgae over 60 min period increased from 441 Wh/g fatty acid to 588 Wh/g fatty acid, as the temperature increased from 60°C to 120°C; additionally, they found that reducing the treatment time to 20 min at 90°C consumed 204 Wh/g fatty acid and did not vary significantly from a higher treatment time. Passos et al. [142] noted an increase in microwave power from 300 W to 900 W resulted in increased temperatures from 50°C to 98°C and that increasing the time from 3 to 9 min at 300 W resulted in temperature increases from 50 to 95°C. Biller et al. [51] found that increasing temperature from 80°C to 140°C resulted in increased microwave power consumption from 25 Wh/g to 61 Wh/g. Azcan and Yilmaz [147] also noted that an increase in microwave temperature radiation consumed higher amounts of energy.

Advantages and disadvantages

Microwave assisted lipid extraction from microalgae is one of the simplest methods and most effective amongst other extraction methods [10]. This technique is moderately suitable for scale up [37]. Microwave microalgae assisted lipid extractions have been noted to be the most applicable method for large scale use due to its simplicity and effectiveness [10]. The rapid extraction time, high heating rates, low operating costs, environmentally friendly nature, lesser solvent requirements, high product purity and high efficiency make it an attractive method for microalgae lipid recovery [28,82,137,138,148]. However, the disadvantages include the maintenance costs associated with large scale applications, an additional step required after pretreatment for lipid recovery, high temperatures can result in lipid degradation and lengthy cooling times are required to avoid lipid loss [28,149].

Biological Pre-Treatment of Microalgae

Enzyme hydrolysis

Enzymes are used to hydrolyze the microalgae cell walls, releasing the intercellular components (such as lipids) into the bulk of the medium, making their recovery much more rapid and effective [24]. Numerous types of enzymes have been reported to be effective in the cell wall degradation of microalgae. These include sanilase, cellulase, neutral protease, alkaline protease, papain and lysozyme (Table 2). Addition of these enzymes to the biomass will work to degrade the polymers on the cell surface which will allow easier recovery of the lipids from the biomass [78,157]. A schematic depicting the microalgae cell wall hydrolysis process is illustrated by Figure 21 [159].

Freshwater microalgae possess highly resistant, non-hydrolyzable aliphatic biopolymers that are made up of even carbon numbered long-chain (30 to 34 carbon atoms) ω-unsaturated ω-hydroxy fatty acid monomers [160]. Intermolecularester links of the monomers form linear chains that act as the starting position of ether cross-linking. These algaenans are highly resistant against degradation as a result of their polyether nature [160-162]. Blokker et al. [160] predicted the structure of these algaenans consisting of linear polyester chains that are cross-linked by ether-bonds as that depicted in Figure 22 [160].

Zheng et al. [29] tested the effectiveness of sanilase assisted cell hydrolysis on *Chlorella vulgaris* biomass and noted a lipid recovery of 7% which was much lower than that achieved using lysozyme and cellulase. Liang et al. [150] reported a lipid recovery of 34% using the enzyme sanilase on microalgae biomass consisting of *Chlorella vulgaris, Scenedesmus dimorphus* and *Nannochloropsis* sp. species. Lu et al. [163] reported that the snailase enzyme was more effective in disrupting *Chlorella protothecoides* cell wall than cellulase as a result of higher proteolysis concentrations achieved. Cheng et al. [8] also noted effective recovery of *Schizochytrium* protoplasts using the combination of snailase and cellulase enzymes for cell wall hydrolysis.

Liang et al. [150] noted that lipid recoveries greater than 30% were achieved from *Chlorella vulgaris* using trypsin and snailase enzymes while lower yields resulted from neutral protease, alkaline protease and cellulase. Sander and Murthy [164] noted that effective cell wall degradation in microalgae cells can be achieved using enzymatic cellulase. Cho et al. [154] noted that the presence of cellulose enzymes increased the cell hydrolysis which resulted in higher solvent lipid recoveries. Fu et al. [156] reported that cellulase enzyme hydrolyses of *Chlorella* sp. increased the lipid recovery to 56% compared to 32% prior to treatment. Liu et al. [152] reported a lipid recovery of 65% from *Chlorella vulgaris* using cellulase assisted enzyme hydrolysis. Zheng et al. [29] reported a lipid recovery of 24% from *Chlorella vulgaris* using cellulase cell wall hydrolysis.

Liang et al. [150] noted that a lipid recovery of 11% using neutral protease microalgae hydrolysis of *Chlorella vulgaris, Scenedesmus dimorphus* and *Nannochloropsis* sp. Liu et al. [152] reported a lipid recovery of 66% from *Chlorella vulgaris* using neutral protease enzyme hydrolysis. Wang et al. [151] noted a cell disintegration that ranged from 10.9% to 24.3% in *Neochloris oleoabundans* using neutral protease corresponding to enzyme concentrations of 1% to 6%. Ying et al. [153] tested neutral protease enzyme extraction of lipids from *Chlorella vulgaris* biomass and noted a recovery efficiency of 85%.

Khomova et al. [165] noted a complete recovery of lipids (37%) from *Monochrysis* biomass using alkaline protease enzyme treatment. Liu et al. [152] obtained a lipid recovery of 66% from *Chlorella vulgaris* biomass using a mixture of alkalai protease and cellulase. Liang et al. [150] noted a lipid recovery of 8% when a mixture of microalgae biomass (*Chlorella vulgaris, Scenedesmus dimorphus* and *Nannochloropsis* sp.) was treated with alkaline protease. Ying et al. [153] reported an extraction efficiency of 80% using enzymatic alkaline protease in *Chlorella vulgaris* biomass.

Wang et al. [151] reported disintegration efficiency in *Neochloris oleoabundans* of up to 45.2% using papain enzyme for cell wall hydrolysis. Horst et al. [166] noted that the papain enzyme assisted cell hydrolysis of *Phaeodactylum tricornutum* biomass was effective in lipid extraction. Morris et al. [167] tested used the enzyme papain to hydrolyze *Chlorella vulgaris* biomass and found that it resulted in a 16% disintegration efficiency. Reddy and Majumder [155] used papain to hydrolyze *Spirogyra* sp. biomass and noted a lipid recovery of 18%.

Gerken et al. [168] noted that the enzyme lysozyme was effective in the digestion of *Chlorella vulgaris* cell wall. Taher et al. [157] reported a lipid recovery from *Scenedesmus* sp. of 16.6% using the enzyme lysozyme for cell wall hydrolysis. Zheng et al. [29] recovered a lipid concentration of 22% from *Chlorella vulgaris* biomass using lysozyme enzyme hydrolysis. Natarajan et al. [78] reported that microalgal cell wall can be effectively degraded using lysozyme enzyme hydrolysis. Cuellar-Bermudez et al. [169] reported that lysozyme was effective in disrupting microalgae cell wall.

Factors affecting enzyme assisted extraction

A number of factors have been noted to influence the efficiency of enzymatic pre-treatment for microalgae lipid extraction. These factors include: treatment time, enzyme concentration, cell concentration, pH and temperature.

Time: Prolonged exposure of enzyme to microalgae biomass allows

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<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Microalgae</th>
<th>Lipid Yield (%)</th>
<th>Reference</th>
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<td></td>
<td><em>Scenedesmus dimorphus</em></td>
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<td>Wang et al. [151]</td>
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</tr>
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<td>Cellulase Novozyme 188</td>
<td><em>Chlorella vulgaris</em></td>
<td>73</td>
<td>Cho et al. [154]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorella vulgaris</em></td>
<td>63</td>
<td>Fu et al. [156]</td>
</tr>
<tr>
<td>Cellulase Papain</td>
<td><em>Neochloris oleoabundans</em></td>
<td>10</td>
<td>Wang et al. [151]</td>
</tr>
<tr>
<td>Lysozyme</td>
<td><em>Scenedesmus sp.</em></td>
<td>16.6</td>
<td>Taher et al. [157]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Scenedesmus sp.</em></td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Lysozyme Cellulase</td>
<td><em>Scenedesmus sp.</em></td>
<td>12</td>
<td>Taher et al. [157]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Nannochloropsis oculata</em></td>
<td>32.7%</td>
<td>Surendhiran &amp; Vijay [113]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorella vulgaris</em></td>
<td>65</td>
<td>Liu et al. [152]</td>
</tr>
<tr>
<td>Cellulase Alkal protease</td>
<td><em>Chlorella vulgaris</em></td>
<td>66</td>
<td>Liu et al. [152]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorella vulgaris</em></td>
<td>35</td>
<td>Zheng et al. [158]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorella vulgaris</em></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Sanilase</td>
<td><em>Chlorella vulgaris</em></td>
<td>24</td>
<td>Zheng et al. [29]</td>
</tr>
<tr>
<td>Lysozyme</td>
<td><em>Chlorella vulgaris</em></td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Enzymes-assisted hydrolysis of microalgae for lipid recovery.

![Figure 21: Schematic depicting enzyme assisted cell wall hydrolysis [159.]](image1)

![Figure 22: Predicted algaenans structure [160.]](image2)
for increased cell wall degradation, Figure 23 [150]. The rupture of microalgae cell wall allows for easier recovery of the intracellular lipids [113,156].

Liang et al. [150] found that lipid yields increased with increasing reaction time from 2 to 12 h for *Chlorella vulgaris*, but reached a plateau at 12 h. Surendhiran and Vijay [113] noted that increasing the reaction time from 8 to 12 h resulted in increased lipid yield from *N. oculata* species. Reddy and Majumder [155] found that increasing the reaction time of enzyme hydrolysis from 1 to 4 h resulted in increased oil yield from 12 to 18%. However further increase in incubation time to 8 h did not show any increases in oil yield and 10 h incubation time resulted in reduction of yield to 17%. Cho et al. [154] noted that increasing the enzymatic reaction time from 1 to 72 h resulted in increased *Chlorella vulgaris* oil yield from 18 to 85%. Fu et al. [156] found that the microalgae lipid content increased with increasing reaction time from 1 to 70 h.

**Enzyme concentration:** The enzyme concentration affects the efficiency of microalgae cell wall degradation. Greater enzyme concentration allows for rapid cell wall degradation by increasing the enzyme to biomass content, Figure 19 [150].

Liang et al. [150] noted that the lipid recoveries from *Chlorella vulgaris* using Trypsin and snailase enzymes at concentrations of 4 to 8% did not vary from one another, but increasing the enzyme concentrations from 0.5 to 2% significantly increased oil yields. Wang et al. [151] noted that increasing the enzyme concentration from 1 to 6% in *Neochloris oleobundans* resulted in increased cell disintegration from 48.2% to 64.4%, 38.1% to 45.2% and 10.9% to 24.3% using cellulose, papain and neutral protease, respectively. Morris et al. [170] noted that increasing the enzyme concentration from 0.55 to 4.5% resulted in increased cell hydrolysis from 10 to 25%. Kose and Oncel [171] found that the highest protein yield, as a result of cell wall hydrolysis, resulted at a biomass concentration of 10 or 15% over the tested range of 5-20%. Kose and Oncel [171] found that the increasing the biomass concentration (lowering the enzyme to biomass ratio) resulted in lower protein yields which is attributed to lower cell wall hydrolysis. Ho et al. [172] noted that increasing the microalgae biomass concentration (10 - 40 g/L) while holding the enzyme concentration consistent, resulted in decreased glucose production yields.

**pH:** The pH of the reaction medium plays an important role in the effectiveness of the enzyme (Table 3) as the function of the enzyme is strongly influenced by the pH, Figure 24 [150].

Cho et al. [154] noted that an optimal pH exists for maximum enzymatic (*Cellulase* and Novozyme 188) hydrolysis of *Chlorella vulgaris* of 4.8 over the tested range of 3.8-5.8. Liang et al. [150] found that snailase and trypsin enzymatic lipid extraction from microalgae was pH dependent and that the increases in pH above 4 (4-9) resulted in lower lipid yields. Fu et al. [156] achieved the highest cell wall hydrolysis in *Chlorella* species at a pH of 4.6 using cellulase over the tested range of 3.6 to 7.6. Harun and Danquah [173] investigated the hydrolysis of *Chlorococcum* sp. using cellulase over the pH range of 2.5 to 7.5 and found an optimal pH between 4 to 6, as a result of enzyme impairment under acidic and alkaline conditions. Reddy and Majumder [155] used papain assisted enzyme cell wall hydrolyses on *Spirogyra* sp. and found it effective at a pH of 6.
Temperature: The temperature of the reaction is crucial for efficient microalgae cell wall degradation. Low temperatures can slow down the reaction rate and high temperatures can result in lipid degradation. Thus the temperature of the reaction must be optimized for the enzymatic activity in order to achieve high lipid recoveries.

Cho et al. [154] noted that a temperature of 50°C resulted in the highest hydrolysis efficiency in *Chlorella vulgaris* over the tested range of 40-60°C. Reddy and Majumder [155] found that increasing the reaction temperature in enzyme hydrolysis from 30 to 60°C resulted in increased oil yields from 14 to 27%, but further increase to 70°C resulted in a decreased oil yield (25%), Fu et al. [156] found that the cell wall hydrolysis rate was highest at a temperature of 50°C over the tested range of 40-60°C in *Chlorella* species. Harun and Danquah [173] found that an optimal temperature of 40°C existed for cellulase activity during over the tested range from 28 to 60°C. Saha and Cotta [174] and Mtui et al. [175] also reported an optimum temperature range of 30-45°C for cellulase cell wall hydrolysis.

**Advantages and disadvantages**

Microalgae lipid extraction using enzymes is a highly specific and rapid which make it desirable for specific biproducts [150,176]. This method requires low operational temperatures and has high specificity/ selectivity of the lipid class and no corrosion issues associated, making it a desirable technique for microalgae cell wall hydrolysis compared to chemical and physical methods [157,177]. However, efficiency is affected by the lipid composition and the type of microalgae [150] and enzymes can be high in cost [28] which limits the use of enzyme hydrolysis on industrial scale [156,178].

**Comparative Analysis**

**Selection of criteria**

Eight criteria (Table 4) were used for the evaluation of microalgae oil extraction pre-treatment techniques: (a) cell wall disruption efficiency, (b) cost, (c) time, (d) suitability for large scale use, (e) toxicity and health, (f) environmental impact, (g) reusability and (h) maintenance. These criteria were selected based on the information reported in the literature regarding the technique. Comparative analysis was performed using these criteria in order to determine the most efficient, cost effective and environmentally friendly microalgae oil extraction pre-treatment technique that is suitable for large scale application.

**Assigning a score to each criterion**

Each of the selected criterions was assigned a score from 10 to 15 which were determined by the degree of importance of the criterion (Table 4). Higher values were given to the criteria that were deemed most important for development of an efficient and economic large scale pre-treatment technique for microalgae biomass. Lower values were given to criteria that were deemed necessary for determining a suitable method but were considered less important. These values were then used to determine the effectiveness of each of the investigated pre-treatment method on microalgae biomass as shown in Tables 5-13.

### Table 3: Optimal pH for enzyme assisted microalgae cell wall hydrolysis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Microalgae</th>
<th>Tested pH range</th>
<th>Optimal pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulase Novozyme 188</td>
<td><em>Chlorella vulgaris</em></td>
<td>3.8-5.8</td>
<td>4.8</td>
<td>Cho et al. [154]</td>
</tr>
<tr>
<td>Snaillase Trypsin</td>
<td><em>Chlorella vulgaris</em></td>
<td>4-9</td>
<td>4</td>
<td>Liang et al. [150]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorella</em> sp.</td>
<td>3.6-7.6</td>
<td>4.6</td>
<td>Fu et al. [156]</td>
</tr>
<tr>
<td>Cellulase</td>
<td><em>Chlorococcum</em> sp.</td>
<td>2.5-7.5</td>
<td>4-6</td>
<td>Harun and Danquah [173]</td>
</tr>
<tr>
<td>Papain</td>
<td><em>Spirogyra</em> sp.</td>
<td>6</td>
<td>-</td>
<td>Reddy and Majumder [155]</td>
</tr>
<tr>
<td>Cellulase Papain</td>
<td><em>Neochloris oleoabindans</em></td>
<td>6.5</td>
<td>-</td>
<td>Wang et al. [151]</td>
</tr>
<tr>
<td>Lysozyme</td>
<td><em>Scenedesmus sp.</em></td>
<td>7.48</td>
<td>-</td>
<td>Taher et al. [157]</td>
</tr>
</tbody>
</table>

**Figure 24:** Effect pH on the lipid recovery from *Chlorella vulgaris* biomass [150].
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Importance</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency</td>
<td>15</td>
<td>The system should be able to effectively disrupt the cell wall of microalgae in order to increase the oil extraction efficiency</td>
<td></td>
</tr>
<tr>
<td>Cost</td>
<td>15</td>
<td>The operational costs of the pre-treatment process should be low, so that the additional inquired pre-treatment costs can be justified</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>15</td>
<td>The rate of microalgae cell wall degradation should be quick to ensure the sustainability purposes</td>
<td></td>
</tr>
<tr>
<td>Suitability for Large Scale Use</td>
<td>15</td>
<td>The method should effective in handling large volumes for industrial production</td>
<td></td>
</tr>
<tr>
<td>Toxicity and Health</td>
<td>10</td>
<td>The method should be non-toxic so that the retrieved algae biomass maybe processed for a number of value added products for animal and/or human consumption</td>
<td></td>
</tr>
<tr>
<td>Environmental Impact</td>
<td>10</td>
<td>Method should be environmentally friendly with no toxic wastes produced</td>
<td></td>
</tr>
<tr>
<td>Reusability</td>
<td>10</td>
<td>The pre-treatment method should be resalable in order to reduce costs associated with equipment</td>
<td></td>
</tr>
<tr>
<td>Maintenance</td>
<td>10</td>
<td>Costs for maintaining the method should be low</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Criteria used for the comparative analysis of different oil extraction pre-treatment techniques.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Effective cell wall disruption of microalgae biomass, but dependent on the likelihood of the contact between cell and bead</td>
<td>10</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>Costs associated with drying of biomass before use and the high energy required for shaking/agitation. Requires cooling jacket in order to avoid degradation of desired product</td>
<td>6</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Rapid</td>
<td>15</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Difficulty in scale up</td>
<td>3</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Maintenance costs associated with bead replacement</td>
<td>6</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>70</td>
</tr>
</tbody>
</table>

Table 5: Evaluation of shaking vessel bead mill microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Effective cell wall disruption of microalgae biomass, but dependent on the likelihood of the contact between cell and bead</td>
<td>12</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>Costs associated with drying of biomass before use and the high energy required for shaking/agitation. Requires cooling jacket in order to avoid degradation of desired product</td>
<td>6</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Rapid</td>
<td>15</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Difficulty in scale up</td>
<td>3</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Maintenance costs associated with bead replacement</td>
<td>6</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>72</td>
</tr>
</tbody>
</table>

Table 6: Evaluation of agitated bead mill microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Effective cell wall destruction method</td>
<td>15</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>Operational costs are associated with high amounts of power</td>
<td>4</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Relatively rapid</td>
<td>12</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Upscale difficulty</td>
<td>4</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be easily reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Minimal costs associated with maintenance</td>
<td>8</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>73</td>
</tr>
</tbody>
</table>

Table 7: Evaluation of horn sonication microalgae pre-treatment.
## Table 8: Evaluation of bath sonication microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Effective cell wall disruption, resulting in increased lipid recovery</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Effectiveness is dependent on the species</td>
<td></td>
</tr>
<tr>
<td>Cost (15)</td>
<td>Relatively low energy requirement</td>
<td>13</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Rapid treatment</td>
<td>14</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Method is suitable for use on industrial scale</td>
<td>15</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be easily reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Little maintenance is required</td>
<td>9</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>93</td>
</tr>
</tbody>
</table>

## Table 9: Evaluation of steam explosion microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Integrity of the cell wall is weakened</td>
<td>12</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>High energy consumption</td>
<td>6</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Prolonged periods of time required</td>
<td>3</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Upscale difficulty</td>
<td>8</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be easily reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Costs associated with pump maintenance</td>
<td>5</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>64</td>
</tr>
</tbody>
</table>

## Table 10: Evaluation of freeze drying microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Integrity of the cell wall is weakened, however not as effective as other cell distribution methods</td>
<td>10</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>High costs associated with high energy consumption required for high heat and pressure</td>
<td>6</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Prolonged periods of time required for effective disruption</td>
<td>3</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Upscale difficulty</td>
<td>8</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be easily reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Costs associated with maintenance can be resistively low</td>
<td>8</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>

## Table 11: Evaluation of autoclave microalgae pre-treatment.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption efficiency (15)</td>
<td>Integrity of the cell wall is weakened</td>
<td>10</td>
</tr>
<tr>
<td>Cost (15)</td>
<td>High costs associated with high energy consumption required for high heat and pressure</td>
<td>6</td>
</tr>
<tr>
<td>Time (15)</td>
<td>Prolonged periods of time required for effective disruption</td>
<td>3</td>
</tr>
<tr>
<td>Suitability for Large Scale Use (15)</td>
<td>Upscale difficulty</td>
<td>8</td>
</tr>
<tr>
<td>Toxicity and Health (10)</td>
<td>No hazardous substances are used</td>
<td>10</td>
</tr>
<tr>
<td>Environmental Impact (10)</td>
<td>No release of harmful compounds or disruption to the environment</td>
<td>10</td>
</tr>
<tr>
<td>Reusability (10)</td>
<td>Method can be easily reused</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance (10)</td>
<td>Costs associated with maintenance can be resistively low</td>
<td>8</td>
</tr>
<tr>
<td>Total (100)</td>
<td></td>
<td>65</td>
</tr>
</tbody>
</table>
Analysis of microalgae pre-treatment techniques

The sum of the scores obtained for the different microalgae pre-treatment techniques are presented in Table 14. The results indicate that of the 9 microalgae pre-treatment methods investigated, 1 mechanical, 1 thermal and 1 electromagnetic technique resulted in scores of 80/100 or greater deeming them efficient and economically suitable for industrial scale. These methods were bath sonication (81), steam explosion (93) and microwave radiation (87). Bath sonication method is effective in cell wall degradation, nontoxic, rapid technique that requires minimal maintenance, but it does however suffer from high operational costs and difficulty in scale up for industrial use. Microwave assisted technology is rapid, effective in cell wall disruption, non-toxic, can be used for large volumes of biomass and the medium maybe reused, but it suffers from high costs associated with maintenance. Steam explosion microalgae pre-treatment is an effective technique for microalgae cell wall rupture allowing the releasing intracellular components, rapid, reusable, relatively low in costs, environmentally friendly and can be used on an industrial scale. However, this method is species specific making it effective for certain microalgae species. Overall, the negative aspects of these three techniques are outweighed by their effectiveness, rapidness and relatively low costs when compared to other pre-treatment techniques. Other mechanical extraction methods suffered from high operational costs, lengthy treatment times, high maintenance costs and the scale up difficulty. Freeze drying and autoclave techniques were deemed unsuitable microalgae pre-treatment techniques because of the high costs, scale up difficulty and long processing times associated. Biological pre-treatment technique are deemed unsuitable as a result of high costs associated with purchasing of enzymes and recovery/separation difficulty after undergoing treatment, long treatment times and high maintenance of enzymes for high efficiency.
Conclusions

Microalgae biomass can be used to produce numerous value added products such as biodiesel, bioethanol, biogas, biophotoreactors, fish feed, animal feed, human food supplements and skin care products. Production of value added products from microalgae biomass requires the growth and recovery of the algae biomass, extraction and downstream processing of the desired product. One of the major obstacles for using microalgae biomass on an industrial-scale, for the production of biodiesel, is the high processing costs. Increasing the lipid recovery efficiency from the microalgae biomass would result in greater product yields (biodiesel). Thus, the aim of this study was to review the current methods used for microalgae pre-treatment and perform a comparative analysis in order to determine the most economically efficient method for large scale use. The effectiveness of the pre-treatment methods investigated was evaluated based on (a) cell wall disruption efficiency, (b) cost, (c) toxicity (d) suitability for large scale use, (e) time, (f) reusability and (g) maintenance. The results indicated that of the 9 microalgae methods investigated 1 mechanical, 1 thermal and 1 electromagnetic radiation technique were suitable. These methods were bath sonication (81), steam explosion (93) and microwave radiation (87). Microwave assisted microalgae pre-treatment technique is rapid, effective in cell wall disruption, non-toxic, can be used for large volumes and the medium maybe reused, but it does however suffer from high maintenance costs. Bath sonication technique is effective in the degradation of cell wall, non-toxic, rapid technique with minimal maintenance required, but suffers from high costs and difficulty in scale up for industrial use. Steam explosion pre-treatment is effective in degrading microalgae cell wall, releasing intracellular components, rapid, reusable, relatively low in costs, environmentally friendly and reusable, but is species specific. Overall, the negative aspects of these three techniques are outweighed by their effectiveness, rapidness and relatively low costs when compared to other pre-treatment techniques. Other mechanical extraction methods suffer from high operational costs, lengthy treatment times, high maintenance costs and the scale up difficulty. Freeze drying and autoclave techniques were deemed unsuitable microalgae pre-treatment techniques because of the high costs, scale up difficulty and long processing times associated. Biological pre-treatment technique were deemed unsuitable as a result of high costs associated with purchasing of enzymes, difficulty in recovery/separation after treatment, long treatment time, and high maintenance required for high efficiency.

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