The Potential of Using Pulsed Electric Field (PEF) Technology as the Cell Disruption Method to Extract Lipid from Microalgae for Biodiesel Production

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Abstract- For the past few years, there has been an explosive growth of interest in biodiesel production from algae based crops. Feedstock from microalgae is a highly promising resource and can be used as an alternative for sustainable and renewable energy since; lipid from microalgae can be converted to biodiesel. The study brief reviews of the processes related to microalgae for biodiesel production. This includes the process of microalgae cultivation, microalgae harvesting, extracting microalgae lipid and conversion of biodiesel from microalgae. Biodiesel yield is dependable on the amount of lipid extracted which is affected by the technology and method of extraction. The microalgae lipid extraction using traditional methods is primarily discussed and followed by the latest technology of microalgae cell disruption based on electroporation concept. Pulsed electric fields (PEF) Technology as the potential method to extract microalgae lipid is proposed in this work. Treatment of PEF associated with conventional extraction, such as solvent extraction is demonstrated to improve the extraction efficiency of lipid and other valuable intracellular components from microalgae. The paper also described the electroporation mechanism occurred in a cell membrane and the factors that affect the mechanism. Several of PEF chamber designs were discussed which adapted from food industries, biotechnology and engineering perspective view. The benefits and limitation of PEF in the microalgae lipid extraction are also mentioned in this work for the purpose of the future improvement of the PEF extraction system.

Keywords Pulsed Electric Field; Microalgae; Lipid Extraction; Electroporation; Biodiesel.

1. Introduction

Currently the level of carbon dioxide (CO_2) in the atmosphere is the highest in human history. It causes the world to warm up and indirectly is blamed for the strange world weather conditions. Gas emissions such as CO_2 released by fuel combustion from vehicles and industrial sectors are the major contributors to greenhouse gases (GHGs) [1]. In fact, the increasing fuel consumption in the transportation industry as well as electricity and thermal

energy generation are directly contributing to the increasing in GHGs emissions [2]. The diminishing fossil fuel supplies, environmental concerns and human health impacts from the use of petroleum fuel have motivated the development of an economic, sustainable and environmental friendly source of feedstock such as biodiesel.

Oil from microalgae had been demonstrated to be the best candidate for biodiesel production and have been extensively mentioned by several authors [2-6]. Microalgae are photosynthetic and aquatic microorganisms that utilized sunlight, water, and CO_2 and convert it to sugar compounds from which biological macromolecules, like lipids, can be produced. Their cell can be classified from prokaryotic to eukaryotic and also the structure can be differentiated by size of a unicellular to multi-cellular [7]. In comparison to the other feedstocks, microalgae as biodiesel feedstock has several advantages such as high growth rates can be cultivated in non-arable land and using non-potable water. Furthermore, the cultivation of microalgae does not displace other food crop cultivation and it can be harvested on a daily basis if cultivated in a semi-continuous or continuous system [6, 7].

Although the cultivation of the microalgae has high potential for biodiesel production, it is offset challenges in the extraction of lipids from the microalgae. Conventionally, microalgae lipids can be extracted by several methods either via chemical or physical extraction. But, finding the most suitable extraction methods of lipid from microalgae is still under development. In this paper, cell membrane electroporation concept specifically, pulsed electric fields (PEF) technology was reviewed. Electroporation method for microalgae lipid extraction is kind of new applications that only a few literatures had been reported related to the studies.

2. Microalgae as the Biodiesel Feedstock

2.1. Cultivation of Microalgae

One of the unique characteristics of microalgae as biofuel feedstock is the microalgae ability to survive in various conditions. Microalgae can be cultivated under photoautotrophic, heterotrophic and mixotrophic culture mode. Photoautotrophic mode defines as the microalgae utilized light source and sequester CO_2 for photosynthesis [8]. In photoautotrophic condition, lipid content and biomass productivity can be increased if additional of CO_2 was supplied to the microalgae [4]. While the heterotrophic culture mode is light independent, but consumed organic source such as glucose and glycerol to reproduce [9]. Microalgae cultivated in heterotrophic culture unable to synthesize its own food, therefore, required an additional nutrient to survive. Mixotrophic culture condition is the combined culture modes of photoautotrophic and heterotrophic.

Microalgae can also be cultivated in open or closed system. Usually, for a large scale production of microalgae biomass, open pond-shallow with paddle wheel to agitate the microalgae was used. One of the major disadvantages of using open system for cultivation is that it highly contaminated because of direct exposed to the surrounding [3]. While for a closed system, photobioreactors (PBRs) were widely used to minimize contamination during the cultivation period. In the work of Mansa et al. [7], it was shown that the maximum cell density and productivity of *Chlorella sp*. can be achieved to 6.01×10^6 cell mL^{-1} and 2.63×10^5 cells mL^{-1} d^{-1}, respectively and by using the outdoor flat plate airlift photobioreactor constructed from fiberglass materials with a volume of 280 L. It is crucial to take note that light requirement is one of the factors that need to be highlighted in the PBR cultivation system. In PBRs the excessive of light intensity that is being supplied to the culture may contribute to photo-inhibition and photo-oxidation, but may affect the limit growth of the culture for low light intensity [5].

2.2. Harvesting of Microalgae Biomass

After the microalgae cultivation, the biomass needs to be harvested before it can be proceed to the extraction process. At this stage, the separation of water from the microalgae or dewatering process is crucial to obtain high concentrated biomass. Unfortunately, dewatering process required high energy. They are several methods of harvesting the microalgae this includes flocculation, centrifugation, filtration, gravity sedimentation and electrophoresis. Flocculation is a harvesting method that aggregates the microalgae cells together to form bigger particles. The bigger the particle size the faster the sedimentation rate [5]. The chemical that causes the microalgae cell to aggregate is called flocculant. Naturally, microalgae cells carry a negative charge that is to avoid from self-aggregation among the cells. The additional of flocculants into the culture, such as natural and synthetic polymers allow the microalgae cells to attach to each other and then form large particles to facilitate the aggregation [2].

Centrifugation is the most popular method for obtaining the concentrated microalgae biomass. The method is widely used in laboratory scale, but not suitable to be used for commercial scale because it is too expensive [4]. Gravity sedimentation is one of the conventional methods to separate water from microalgae and it is very commonly used in waste-water treatment. The method is very simple, but it is very time consuming [5]. In additional, the method is limited to microalgae with size larger than 70μm [2].

2.3. Extraction of Lipid from Microalgae Biomass

Based on the analysis reviewed by Mata et al. [10], the lipid productivity and lipid content of microalgae from freshwater and marine microalgae can achieve from 11.2 to 142 mg L^{-1} day^{-1} and 4 to 75% dry weight basis, respectively. Different microalgae species produced different levels of lipid and fatty acid composition. Lipids in microalgae can be divided to neutral lipids, which store at lipid granule for energy purpose and polar lipids that mostly found in the bilayer of the cell membrane. Neutral lipids consist of acyglycerols and free fatty acids, whereas polar lipids contain phospholipid and glycolipids [11].

The lipid extraction efficiency can be defined via the disruption methods and type of equipment that is being utilized. The higher extracted lipids lead to higher biodiesel yield. Hence, it is crucial to select the most suitable cell

disruption methods and technologies for attaining higher extraction yields. Microalgae lipid extraction using the electric shock method either direct current, alternating current or electric pulse has been reported for the past four years. In this study, a brief explanation of conventional lipid extraction and the recent technology mainly focused on pulsed electric field is discussed details in section 3. Using PEF as the lipid extraction method for microalgae is relatively a new method. There were only a few literatures that have been published regarding the microalgae lipid extraction using PEF alone [12-16] and PEF assisted extraction with solvent extraction [17-21].

2.4. Conversion of Biodiesel from Microalgae Lipid

The lipid of microalgae can be converted to biodiesel via transesterification (lipid extraction first conducted, followed by transesterification) or in situ transesterification (lipid extraction of alcohol and transesterification occurred at the same time). Biodiesel or fatty acid alkyl ester is formed based on the chemical reaction of triglycerides and alcohol with the help of a catalyst such as sodium hydroxide (NaOH) which also produced glycerol as the side product. Figure 1 shows the general chemical reaction of biodiesel formation. The radicals R_1, R_2 and R_3 are representing the long chain of hydrocarbons which is the fatty acids.

Microalga *Chlorella sp*. consist of highly saturated fatty acids such as palmitic acid and stearic acid which are the common fatty acids in biodiesel compound that actually promotes a good certain number and oxidative stability to biodiesel [22]. In theory, the molar ratio of oil to alcohol is 1 to 3 and 1 kg of microalgae oil will produce 1 kg of biodiesel (esters) and 1kg of glycerol as the side product as illustrated in Fig.1. They are several factors that need to be considered during the transesterification process, these include the reaction of time, type of catalyst used (acidic, basic or enzymatic), concentration of catalyst, alcohol portion and the reaction temperature [23].

Several literatures had also reported on the improvement of biodiesel yields. Dong et al. [24] had found that two-step in situ transesterification using Amberlyst-15 with potassium hydroxide (KOH) as the catalysts were capable to increase 3.5 times of fatty acid methyl ester (FAME) recovery percentage higher than using in situ transesterification of KOH alone. Amberlyst-15 was an acidic catalyst and used as pre-esterification to convert the lipid of *Chlorella sorokiniana* (UTEX 1602) to FAME. Followed by the basic catalyst which the KOH for transesterification process.

A recent study conducted by Teo et al. [25] had found that lipase from Candida rugosa (Type VII) provided 7 times greater of biodiesel yield from marine microalga *Testraselmis sp*. than via transesterification using alkali based catalyst. One of the advantages of using transesterification via enzymatic methods such as lipase is the reaction temperature lower than that of reaction temperature of alkali or basic based catalyst.

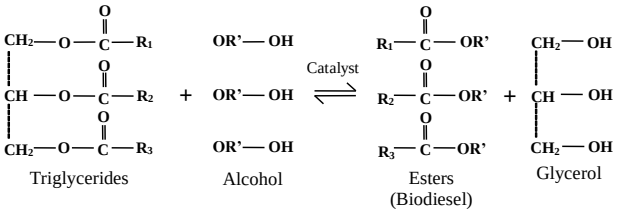

Fig. 1. Transesterification process in biodiesel formation.

3. Lipid Extraction Method from Microalgae Biomass

3.1. Conventional Methods for Microalgae Lipid Extraction

The major challenge for lipid extraction from microalga is to break its cell wall. Microalgae appear to have a variety of sizes from few 2 µm to 100 µm depending on their species which cannot be viewed with naked eyes. The microalgae lipid is stored in lipid granule, therefore in order to extract the lipid the cell wall of microalga needs to be lysed or disrupted. Microalgae cell walls are made from carbohydrates [26] and glycoproteins [27].

The cell wall was very thick and rigid due to the existence of covalent bonds, hydrogen bonds and van der Waals forces interaction among the molecules [28]. Conventionally, cell disruption of microalgae can be divided into mechanical and non-mechanical methods. Non-mechanical included physical, chemical and enzymatic. The various cell disruption methods for single and combined methods are all summarized in Table 1.

3.1.1. High Pressure Homogenizer

High pressure Homogenizer (HPH) is classified as a mechanical cell disruption method. The method was found to be very effective to break the microalgae cell wall. The microalgae cells were pumped and forced to pass a narrow orifice using high pressure around 2 kPsi to 45 kPsi (Fig.2) resulting the sample or fluid in high velocity jet [29, 30]. Hence, shear forces created from the homogenizer nozzle and facilitate the rupture of the microalgae cell wall. The shear forces are influenced by the parameters such as the fluid viscosity, size of the nozzle and the amount of pressure used in the homogenizer [29]. One of the advantages using homogenizer for cell rupture is its ability to be scaled up for microalgae lipid extraction.

Halim et al. [30] had reported that, the microalga *Chlorococcum sp*. cell can be disrupted about 73.8% of initial intact cells using the pressure of 12.33 kPsi (850 bar) with five numbers of passes. Spiden and co-workers had conducted a study on the microalgae cell disruption using HPH for three different species of microalgae.

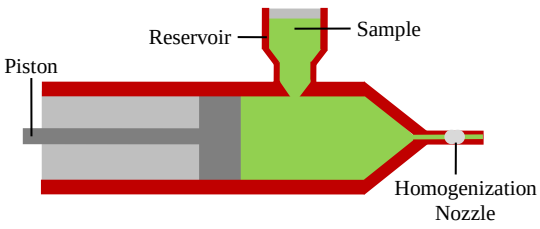

Fig. 2. High Pressure Homogenizer (Adapted from Samarasinghe et al. [29]).

The study found that, different species required a different pressure of cell rupture, which *Nannochloropsis sp*. required the highest pressure (29 kPsi) for achieving 50% of cell ruptured. This followed by the microalga *Chlorella sp*. with 7.38 MPsi and *Tetraselmis sp*. with 1.17 MPsi [31]. According to Samarasinghe et al. [29] the cell wall of microalga *N. oculata* can be effectively ruptured at a pressure of 10 kPsi for two passes through 195 μm homogenizing nozzle and the size of nozzle does not affect the cell wall damage of the microalga.

3.1.2. Sonication

A mechanical cell disruption method using sonic waves with broad frequency like sonication is also a popular method for microalgae lipid extraction. The sonic waves create an enough cavitation impact on the cell wall and membrane that causes the cell to be disrupted. There are two types of sonicator, they are horns and bath type as described by Lee et al. [28]. The horn type of sonicator utilizes piezoelectric generator while bath type of sonicator utilizes transducers to create ultrasonic waves.

A current study implemented by Wang et al. [32] using a high frequency focused ultrasound (HFFU) and low frequency non-focused ultrasound (LFNFU) performance towards lipid extraction from both microalgae *S. dimorphus* and *N. oculata*. Figure 3 illustrates the two types of devices used in their studies Fig.3a is the HFFU and Fig.3b is the LFNFU device. The HFFU operated at 3.2 MHz with input power of 40 W, while LFNFU operated at 20 kHz with input power of 100W. The purpose of using an ice bath on both methods was to absorb the heat generated by the ultrasonic waves. The study shows that using the HFFU device increased the lipid rate % of *S. dimorphus* to 1.2 times greater than using the LFNFU device for 5 minutes of treatment. In contrast, using the LFNFU device increase the lipid rate % of *N. oculata* to 1.8 times greater than using the HFFU device for similar treatment time. The HFFU device consumed less energy compared to the LFNFU device. However, the combination of HFFU and LFNFU found to improve the lipid rate percentage [32].

Other than extracting the lipid, sonication also been used for carbohydrate extraction. The microalgae cell walls enrich with carbohydrate content can be hydrolyzed and convert to glucose using yeast for fermentation of bio-alcohol. The carbohydrate content can be increased by using ultrasonic as a pre-treatment method prior to hydrolysis treatment [33].

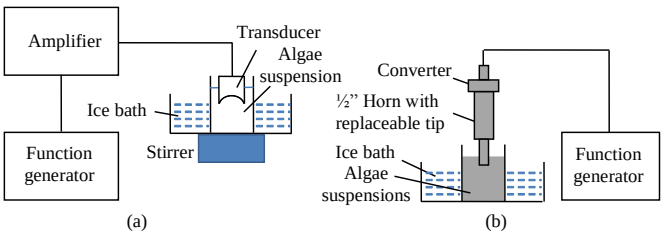

Fig. 3. The schematic diagram of ultrasonic devices (a) High frequency focused ultrasound, (b) low frequency non-focused ultrasound (modified and adapted from Wang et al. [32]).

Neto et al. [34] used sonication bath as the prelude of the solvent extraction method to extract lipid from three microalgae species. Based on the results attained from the study, the lipid extracted from *T. fluviatilis* and *T. pseudonana* have almost the same % dry weight of 40.3% and 39.0 % respectively. While for *C. minutissima* the extracted lipid obtained was the lowest with 15.5% of dry weight. Araujo et al. [35] have also agreed that, ultrasound assisted extraction (UAE) enhanced the lipid extraction of *C. vulgaris*. They used ultrasonic bath, type sonicator to disrupt the *C. vulgaris* cell with the operating condition at 40 kHz and 2.68 W/m^2 of the frequency and ultrasonic intensity, respectively. Solvent extraction using the Bligh and Dyer method was employed in the study and attained 52.5% wt of extracted lipid.

A study conducted by Dey and Rathod [36] using UAE to extract β-carotene from *Spirulina platensis*. In order to avoid the erosion of the tip and reduce the energy consumption, the experiment was performed in pulse mode rather than operating in continuous mode. The effects of duty cycle and electrical acoustic intensity on the UAE performance extraction were the two of the parameters used in the study. The elevation of duty cycle from 0% to 61.5% increased the extraction yield of *β*-carotene ≈1.10 mg/g. The extraction yield also increased ≈ 1.04 mg/g if using the intensity of 64 to 167 W/cm^2. Despite, the UAE method able improves the extraction yield, the method was less efficient compared to other extraction methods such as HPH, bead-beating and sulphuric acid treatment [30].

3.1.3. Microwave Assisted Extraction

A physical conventional method like microwave also imparts an effective of microalgae cell disruption. The method is a non-contact heat source and it has been used for the last 10 years in microalgae lipid extraction [37]. Microwave project a high frequency wave to heat the target sample that attributed by an electromagnetic field which cause the ions in the solution to vibrate and the dipolar molecules to rotate [38].

A work employed by Cheng et al. [39] on lipid extraction of wet microalga *C. pyrenoidosa* for direct biodiesel production via microwave irradiation. They used the single-step method which the lipid extraction and transesterification occurred at the same time and compared with traditional method. The study found that almost 77.5% of *C. pyrenoidosa* cell walls were disintegrated. Based on their findings also, the biodiesel yield achieved 1.3 times greater than that of using conventional heating and two-step processes which the lipid extraction and transesterification conducted step by step. However, the combination of microwave and solvent extraction were also being applied and reported. The solvent was performed subsequently to microwave for pre-treatment. Due to the microwave capability to disrupt the thick and rigid cell wall of microalgae, this gives the solvent to be able to penetrate easily deep inside the cell and extract the lipid.

An interesting study performed by Iqbal and Theegala [40] using microwave and biodiesel (methyl soyate) as the co-solvent for lipid extraction of *Nannochloropsis sp*.. Normally, the types of solvents used for extraction process

were methanol, chloroform, ethanol and n-hexane, but in their studies biodiesel was performed prior to conventional solvents. This can be explained that conventional solvents are highly toxic for the environment and must be reduced. It has been proven that the oil extraction efficiency can be enhanced 115.5% with the higher usage of biodiesel as co-solvent (40% biodiesel and 60% ethanol) at high temperature condition (120˚C) which was comparable with the conventional Soxhlet extraction method [40].

Lee et al. [41] had compared several extraction methods such as microwave, autoclaving, bead-beating, sonication and an osmotic shock (10% NaCl solution) for microalga lipid extraction. It was elucidated that, the microwave extraction method is simpler, easier, less time consuming and provide an efficient result.

3.1.4. Osmotic Shock

The osmotic shock technique also been employed in microorganism cell disintegration. The technique used an osmotic agent such as glucose, sodium chloride (NaCl) and sorbitol (a sugar alcohol) to disrupt the rigid cell walls of the microorganism. The cells are placed in a high osmotic pressure medium to make the cells in an equilibrium state. After the cells are equilibrated, water is added for dilution. The sudden dilution causes the water to enter the cell quickly and lead its internal pressure to increase. At this state, the cells will be disrupted or lysed due to the difference of osmotic pressure on the internal and outside of the cell membrane [42].

According to Yoo et al. [43] lipid from wet *C. reinhardtii* biomass can be recovered almost 2-fold using osmotic shock associated with solvent extraction. In terms of the lipid extraction efficiency, Lee and co-workers had demonstrated that osmotic shock method is comparable to bead-beating method for lipid extraction from *C. vulgaris* and *Scenedesmus sp*. [41]. One of the drawbacks using osmotic shock for lipid extraction application is time consuming [41]. On the other hand, similar to the microwave extraction method, the osmotic shock also simple and easy to conduct.

3.1.5. Solvent Extraction

Solvent extraction method is the most common method for microalgae lipid extraction due to simpler process and less time consuming. It is also known as liquid-liquid extraction system. The combination of solvents for lipid extraction invented by Folch et al. [44] and Bligh and Dyer [45] are the most popular methods to extract lipid from the biomass, so far [46-49]. During microalgae lipid extraction, the mass transfer happens from the inside to the outside of the microalgae cell wall. The inside mass refers to the lipid droplets of microalgae then will be extracted by the solvent system [40].

Recently, Teo and Idris [49] have studied and compared the performances of the solvent extraction from the well-established Folch, Bligh and Dyer, Hara and Radin, and Chen method with the assisted microwave irradiation method. The Bligh and Dyer method used the solvent mixture of chloroform, methanol and water, while the Chen method utilizes methanol and dichloromethane. The Folch method uses similar solvent as Bligh and Dyer method except the Floch method do not use water in the solvent system. The Hara and Radin method was quite different than the other three methods, which use isopropanol and hexane. Marine microalgae *Nannochloropsis sp*. and *Tetraselmis sp*. were chosen for the lipid extraction candidates. The results had suggested that, the Hara and Radin method was the best method to extract the lipid from *Tetraselmis sp*. with 8.19 % (volume extracted lipid/algae dry weight) as for *Nannochloropsis sp*. higher lipid extracted percentage obtained from Folch method with 8.47%. Hence, different microalgae species will respond to the different solvent extraction system.

Ryckebosch et al. [50] had reported that the solvent mixture of hexane and isopropanol with a ratio of 3 to 2 performed better than that of hexane alone. They had also attained the lowest percentage of lipid extraction from both *Nannochloropsis* species, which agreed the results gained from Teo and Idris [49]. Nevertheless, using hexane-based oil extraction system for large scale production of algae-biodiesel demonstrated to be the most compatible with the view of energy analysis, such as low cost, less complicated and low energy consumption [51].

In the work of Ramluckan et al. [52], variety of solvents such as petroleum ether, hexane, cyclohexane, isooctane, toluene, benzene, diethyl ether, dichloromethane, isopropanol, chloroform, acetone, methanol and ethanol were used to extract the *Chlorella sp*. lipid. Unlike the above result mentioned, using chloroform as single extraction gave the high extraction efficiency with 10.78% and solvent mixture with 1 to 1 ratio of chloroform and ethanol shown good extraction yield of 11.76% for Soxhlet extraction method. The optimum treatment time was found to be 3 hours.

Nevertheless, one of the major concerns of utilizing solvent as lipid extraction method is the impact on human health and high toxicity such as chloroform and n-hexane. Moreover, several of the solvents are highly flammable for example, methanol, ethanol, etc. Therefore, it is important to develop a solvent system with less harmful, inexpensive and environmentally friendly.

3.1.6. Supercritical Fluid Extraction

Supercritical fluid extraction was found to be one of the conventional extraction methods that also promote higher extraction efficiency. If gas such as CO_2 was heated and compressed at its critical points, the physical properties of the gas will change dramatically and the condition so-called supercritical fluid. In this state, both gas and liquid phase are coexisted and it appears to gain the solubility power of a liquid and diffusivity of a gas. Supercritical CO_2 (SC-CO_2) extraction method gives yield with high quality, which the extracted compounds will not be contaminated and deteriorated [4]. Moreover, CO_2 is a non-toxic solvent, non-flammable and environmentally friendly.

Cheng et al. [53] had conducted a study on the lipid extraction from microalga *Pavlova sp*. using SC-CO_2 extraction. The extraction system was operated at 306 bar and 60˚C for 6 hours of extraction time. Bead-beating

machine was used as pre-treatment to disrupt the microalga cell wall. The results indicated that, the combination of extraction method using bead-beating and SC-CO_2 achieved the highest percentage of FAME with 98.7% compared to SC-CO_2 extraction alone.

A study conducted by Mouahid et al. [54], using the mathematic modelling developed by Sovová [55, 56], specifically to extract valuable compounds from plant and vegetables. They used microalgae *N. oculata*, *C. closterium*, *C. vulgaris* and *S. platensis* and it was proven that the Sovová's modelling equation was applicable for microalgae sample, but it was only recommended to be applied to a high number of experiments. Figure 4 illustrates the schematic diagram of a supercritical CO_2 extraction system for laboratory scale [54]. Dry sample of microalgae will be placed in the extraction autoclave. The CO_2 will be pre-heated before it enters to the extraction autoclave. The purpose of the expansion valve is to maintain the pressure and flow at a constant value. They had obtained more than 90 % weight of triglycerides from the four microalgae, by using the SC-CO_2 extraction operating condition of pressure, temperature and CO_2 flow rates with 40 MPa, 333 K and from 0.3 to 0.5 kg h^{-1} respectively.

Regards on the limitation of using SC-CO_2 extraction, the extraction method is very time consuming, energy intensive and not yet suitable to be applied to large scale production. Samarasinghe et al. [29] have also stated that, in order obtained high yields, the sample that need to be extracted must undergo pre-treatment prior to SC-CO_2 extraction which definitely increases the costs for processing.

3.1.7. Enzymatic

Apart from physical and chemical method of cell disruption, enzymatic technique is also considered as an alternative method and the method consumes less energy compared to the mechanical methods. The enzymes are capable to degrade or deconstruct the cell wall of the microorganism [42, 57]. For example, lysozyme suitable for degrading a cell wall that has peptidoglycan compounds, whereas an isolated enzyme from *Cytophaga* can disrupt the cell wall of *Saccharomyces cerevisiae* [42]. By all means, the selection of enzymes is highly dependable with the target cell wall compositions made of from the microorganism.

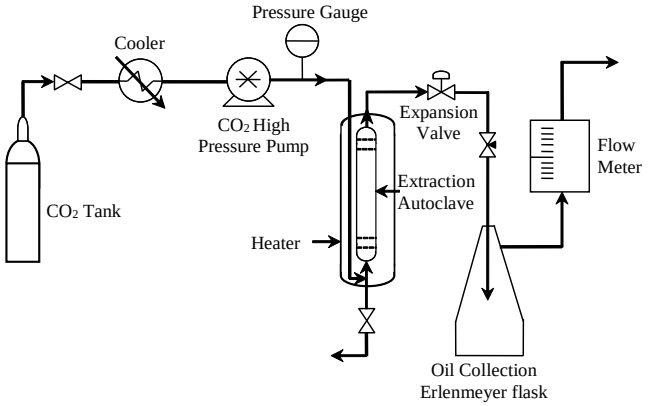

Fig. 4. The schematic diagram of supercritical CO_2 extraction system (Adapted from Mouahid et al. [54]).

Horst and co-workers have studied the various enzymes such as viscozyme, driselase, crude papain, lipase from *Rhizomucor miehei* and Proteinase K. Viscozyme (multi-enzyme mixture composed a wide range of carbohydratases) on the cell wall degradation of marine microalgae *P. tricornutum*, *T. pseudonana*, and *N. oculata*. The study was aimed to investigate the suitable candidates of the enzyme to be used for each microalga. The results elucidated that viscozyme, Proteinase K and crude papain were suitable for *P. tricornutum*. As for *T. pseudonana* using Proteinase K. and crude papain for enzyme-cell wall degradation, while *N. oculata* cells best treated with viscozyme and Proteinase K. [57].

The *Chlorella sp.* cell can be lysed enzymatically using immobilized cellulose (supported by polyacrylonitrile nanofibrous membranes) associated solvent extraction using n-hexane. The enzyme found to be potential for degrading the cell wall with 62% of disruption at temperature of 50˚C and a pH of 4.6 using 20 g/L of microalga. Besides that, the enzymatic method had demonstrated to enhance the extraction efficiency to 1.75-fold (56%) higher compared to the untreated microalga (32%). It also reported that, the immobilized cellulose still can be reused after utilized five times, thus reduce the production cost [58].

Even so, the costs of enzymes are very expansive and a precise enzyme is required to disrupt the cell wall of specific microalgae. Since different microalgae species are constructed with different cell wall composition [57]. Hence, it is still not feasible for commercial scale.

3.2. Novel Methods for Microalgae Lipid Extraction

A recent work by Daghrir et al. [59], based on the cell electroporation concept using electric field treatment to disrupt the cell wall of *C. vulgaris* for lipid and protein extraction. The cell membrane electroporation will be described in details in section 4. The method used by Daghrir and co-workers was adapted from the electrolysis method which was normally applied in microalgae harvesting technique to attain a concentrated biomass [60].

The experiment had been demonstrated in a laboratory scale using a treatment chamber fabricated from Plexiglass material with a volume capacity of 1275 cm^3. Stainless steel (SS) plate was used as cathode electrode, whereas Titanium (Ti), Titanium/Iridium (IV) Oxide (Ti/IrO_2), Titanium/Tin (IV) Oxide (Ti/SnO_2) and Titanium/Lead (IV) Oxide (Ti/PbO_2) were used as anode electrode. By means, the cathode electrode remained the same, while the anode electrodes were varied. Parameters such as the gap between electrodes, surface area for each electrode, current intensity and treatment time were remained constant to 1.0 cm, 110 cm^2, 0.3A and 60 minutes respectively. 0.01mol/L of Sodium sulphate (Na_2SO_4) was added into the mirage solution to enhance the solution electrical conductivity.

Figure 5 illustrates the schematic diagram of the experiment. They had gained the highest extracted lipid with 3.27% wt for Ti/IrO_2 and SS at anode and cathode electrode respectively. This followed by Ti/SnO_2, Ti and Ti/PbO_2 with

Table 1. Summary of conventional cell disruption methods

Classifications of disruption method	Type of cell disruption method	Assisted extraction (after cell disruption)	Microalga strain	Operating condition	Cell disruption (%)	Lipid extraction (% wt)	Ref.
Mechanical	HPH	-	*Chlorococcum sp*.	500 or 850 bar, 5 passes, 15 mins	73.8	-	[30]
	HPH	-	*Tetraselmis sp.*	170 bar, 1 pass	50	-	[31]
		-	*Chlorella sp.*	1070 bar, 1 pass	50	-	
		-	*Nannochloropsis sp*.	2000 bar, 1 pass	50	-	
	HHFU	-	*Nannochloropsis oculata*	3.2 MHz, 40W, 5 mins	-	27.3[a]	[32]
		-	*Scenedesmus dimorphus*	3.2 MHz, 40W, 5 mins	-	112.7[a]	
	LFNFU	-	*Nannochloropsis oculata*	20 kHz, 100W, 5 mins	-	48.6[a]	
		-	*Scenedesmus dimorphus*	20 kHz, 100W, 5 mins	-	96.4[a]	
	US	-	*Chlorococcum sp*.	40 kHz, 65W or 130 W, 25 mins	4.5	-	[30]
	US	-	*Chlorella sp.*	20 kHz, 1000W, 40 mins	97.9[b]	-	[33]
		-		20 kHz, 800W, 40 mins	89.7[b]	-	
		-		20 kHz, 1000W, 40 mins	77.5[b]	-	
	US	Solvent extraction	*Chlorella minutissima*	Ultrasonication ice/water bath during 20 mins (The condition not specified by the authors), n-C_6 H_{14}	-	15.5	[34]
			Thalassiosira fluviatiis		-	40.3	
			Thalassiosira pseudonana		-	39.5	
	US	Solvent extraction (Bligh and Dyer method)	*Chlorella vulgaris*	40 kHz, 2.68 W/m^2, 25˚C (condition same for the 1st and 2nd extraction except the treatment time) *1st extraction:* CH_3OH : $CHCl_3$: H_2O (5:2.5:1; v/v/v) Sonicated for 40 mins *2nd extraction:* $CHCl_3$: Na_2SO_4 (1:1; v/v) Sonicated for 20 mins	-	52.5	[35]
		Solvent extraction (Chen method)		40 kHz, 2.68 W/m^2, 25˚C (condition same for the 1st and 2nd extraction except the treatment time) *1st extraction*: CH_3OH, Sonicated for 3 mins *2nd extraction*: CH_2Cl_2, Sonicated for 27 mins	-	10.9	

	US	Solvent extraction (Folch method)	*Chlorella vulgaris*	40 kHz, 2.68 W/m^2, 25˚C (condition same for the 1st and 2nd extraction except the treatment time) *1st extraction*: CH_3OH, Sonicated for 3 mins *2nd extraction*: $CHCl_3$, Sonicated for 27 mins	-	16.1	[35]
		Solvent extraction (Hara and Radin method)		40 kHz, 2.68 W/m^2, 25˚C (condition same for the 1st and 2nd extraction except the treatment time) *1st extraction*: C_3H_7OH, Sonicated for 4 mins *2nd extraction*: C_6H_{14}, Sonicated for 56 mins	-	2.2	
		Soxhlet method		40 kHz, 2.68 W/m^2, Soxhlet extraction for 8 hrs using acetone	-	1.8	
	Microwave Irradiation[c]	Solvent extraction	*Chlorella pyrenoidosa*	2.45 GHz, 400 W, 30 mins, CH_3OH : $CHCl_3$ (1:1; v/v)	77.5	19.03	[39]
	Microwave Irradiation	Solvent extraction (Bligh and Dyer Method)	*Nannochloropsis sp.*	500 W, 5 mins, 65 ˚C (condition same for the 1st and 2nd extraction) *1st extraction*: CH_3OH : $CHCl_3$: H_2O (5:2.5:1; v/v/v) *2nd extraction*: $CHCl_3$: Na_2SO_4 (1:1; v/v)	-	4.2[d]	[49]
			Tetraselmis sp.		-	7.4[d]	
		Solvent extraction (Chen Method)	*Nannochloropsis sp.*	500 W, 5 mins, 65 ˚C (condition same for the 1st and 2nd extraction) *1st extraction*: CH_3OH *2nd extraction*: CH_2Cl_2	-	8.1[d]	
			Tetraselmis sp.		-	6.8[d]	
		Solvent extraction (Folch Method)	*Nannochloropsis sp.*	500 W, 5 mins, 65 ˚C (condition same for the 1st and 2nd extraction) *1st extraction*: CH_3OH *2nd extraction*: $CHCl_3$	-	8.4[d]	
			Tetraselmis sp.		-	5.4[d]	
		Solvent extraction (Hara and Radin Method)	*Nannochloropsis sp.*	500 W, 5 mins, 65 ˚C (condition same for the 1st and 2nd extraction) *1st extraction*: C_3H_7OH	-	5.9[d]	

	Microwave Irradiation		*Tetraselmis sp.*	*2nd extraction*: C_6H_{14}	-	8.2[d]	[49]
	Microwave Accelerated Reaction System	Solvent extraction	*Nannochloropsis sp.*	2.45 GHz, 1.2 kW, 15 mins, 100˚C, (20% Methyl Soyate in C_2H_5OH)	-	16.5	[40]
				2.45 GHz, 1.2 kW, 15 mins, 120˚C, (40% Methyl Soyate in C_2H_5OH)	-	56.6	
				2.45 GHz, 1.2 kW, 15 mins, 120˚C, $CHCl_3$: C_2H_5OH (1:2; v/v)	-	53.1	
	Microwave Oven	Solvent extraction	*Botryococcus sp.*	2450 MHz, 5 mins, 100 ˚C $CHCl_3$: CH_3OH (1:1; v/v)	-	≈ 39.0	[41]
			Chlorella vulgaris.		-	≈ 10.0	
			Scenedesmus sp.		-	≈ 11.0	
	Osmotic Shock	Solvent extraction	*Botryococcus sp.*	10% NaCl Solution, vortex for 1 min and maintained for 48 hrs, $CHCl_3$: CH_3OH (1:1; v/v)	-	≈ 11.0	[41]
			Chlorella vulgaris.		-	≈ 8.0	
			Scenedesmus sp.		-	≈ 8.0	
	Osmotic Shock	Solvent extraction	*Chlamydomonas reinhardtii* (wildtype)	60 g/L of NaCl, C_6H_{14} : CH_3OH (7:3; v/v)	-	6.67[e]	[43]
				60 g/L of sorbitol, C_6H_{14} : CH_3OH (7:3; v/v)	-	5.61[e]	
			Chlamydomonas reinhardtii (cell wall-less Mutant)	60 g/L of NaCl, C_6H_{14} : CH_3OH (7:3; v/v)	-	9.88[e]	
				60 g/L of sorbitol, C_6H_{14} : CH_3OH (7:3; v/v)	-	5.96[e]	
	-	Solvent extraction	*Isochrysis galbana*	$CHCl_3$: CH_3OH (1:1; v/v)	-	27.7[f]	[50]
			Nannochloropis gaditana		-	28.2[f]	
			Nannochloropsis sp.		-	30.2[f]	
			Phaeodactylum tricornutum.		-	17.8[f]	
	-	Solvent extraction	*Chlorella sp.*	61.5 ˚C, 3 hrs, $CHCl_3$	-	7.26	[52]
				78.0 ˚C, 3 hrs, C_2H_5OH	-	9.40	
				69.0 ˚C, 3 hrs, C_6H_{14}	-	4.81	
				61.5 to 78 ˚C, 3 hrs, $CHCl_3$: C_2H_5OH (1:1; v/v)	-	11.76	
	-	Solvent extraction	*Pavlova sp.*	69.0 ˚C, 15 hrs, C_6H_{14}	-	13.5	[53]
				69.0 ˚C, 100 hrs, C_6H_{14}	-	18.5	

	Bead-beating			Bead beating: 1500 rpm, 5 mins. Soxhlet extraction: 69.0 ˚C, 15 hrs, C_6H_{14}	-	15.3	
	-	SC-CO_2	*Nannochloropsis oculata*	40 MPa, 60 ˚C, CO_2 flow rates from 0.3 to 0.5 kg h^{-1}	-	93.82[g]	[54]
	Bead-beating	SC-CO_2	*Pavlova sp.*	Bead beating: 1500 rpm, 5 mins. SC-CO_2 extraction: 306 bar, 60 ˚C, 6 hrs	-	17.9	[53]
	-	SC-CO_2		306 bar, 60 ˚C, 6 hrs,	-	10.4	
Enzymatic (Non-mechanical)	Enzyme	-	*Phaeodactylum tricornutum*	1 mg/ml of Viscozyme, 37 ˚C, 2 hrs	-	92[h]	[57]
		-		1 mg/ml of Papain, 37 ˚C, 2 hrs	-	104[h]	
		-		1 mg/ml of Proteinase K., 37 ˚C, 2 hrs	-	100[h]	
		Solvent extraction		Enzymatic: 1 mg/ml of Papain, 37 ˚C, 2 hrs Solvent Extraction: C_7H_{16} : C_3H_7OH (3:2 ; v/v)	-	96[i]	
		Solvent extraction		Enzymatic: 1 mg/ml of Papain, 37 ˚C, 2 hrs Solvent Extraction:C_7H_{16}	-	56[i]	
		-	*Thalassiosira pseudonana*	1 mg/ml of Driselase 37 ˚C, 2 hrs	-	100[h]	
		-	*Nannochloropsis oculata*	1 mg/ml of Viscozyme 37 ˚C, 2 hrs	-	88[h]	
		-		1 mg/ml of Proteinase K. 37 ˚C, 2 hrs	-	89[h]	
	Enzyme	Solvent extraction	*Chlorella sp.*	Enzymatic: Immobilized cellulase 60 ˚C, 4.6 pH, 72 hrs Solvent extraction: n-C_6H_{14} , 28˚C	62[j]	10.6[k]	[58]

Note:

HPH – High pressure homogenizer; US – Ultrasonicator/Ultrasound; HFFU - High frequency focused ultrasound; LFNFU - Low frequency non-focused ultrasound; SC-CO_2 – Supercritical carbon dioxide extraction

[a] Relative lipid increase rate (%) = (Lipid fluorescence density of the treatment - Lipid fluorescence density of the control) / (Lipid fluorescence density of the control) x 100%

[b] Cell breakage rate (%) = (The total cells before treatment – Cell left after treatment) / (Total cell before treatments) x 100 %

[c] One-step method refers the simultaneous extraction and transesterification processes

[d] Yield of lipid extraction efficiency (% v/w) = (Volume of extracted lipid) / (Cell dry weight in each sample) x 100 %

[e] Lipid yield is expressed in mg/l. [f] Total lipid content is expressed in g/100 g dry weight. [g] Total of triglycerides in % mass (biomass of 2 to 13 grams of dry weight)
[h] Lipid amount in % (quantified using the Nile Red assay). [i] The amount of Triacylglycerol (TAG) in %, based on the standard lipid extraction using the solvent mixture of $CHCl_3$ and CH_3OH (2:1; v/v). [j] Cell disruption % based on hydrolysis yield (microalga cell wall degraded). [k] Lipid concentration in mg/ml

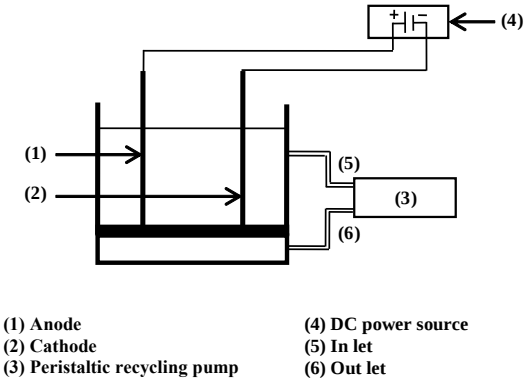

Fig. 5. The schematic diagram of the electrochemical treatment (Adapted from Daghrir et al. [59]).

extracted lipid of 2.76, 2.65 and 2.08 % wt, respectively (see Table 2). Anode electrode made from Ti/IrO_2 has the advantages such as lower crystalline nature than that of SnO_2 and stronger interaction between hydroxyl and the electrode surface compared to Ti/PbO_2.

The results clearly elucidated that, the type of materials used for anode electrode affects the lipid extraction efficiency. It is crucial to take note that, the above technique is using lower voltage, thus long time of treatment is required in order to get high yields. Moreover, the conductivity between the anode and cathode electrode is also affecting the lipid yield, good conductivity creates high electric field strength. The study also found the optimal parameters for the electrochemical treatment using the factorial design methodology. Based on the experimental design method, the recycling flow rate of the microalga solution contributed the highest respond with 61.72%, followed by current intensity by 22.63% and electrolysis time with 14.15% [59]. The recycling flow rate can be correlated with the increasing of mass transfer coefficient. Such method is potential to be scaled up. Nevertheless, the improvement of the electrochemical system, for example volume of the sample, continuous system and treatment time should be taken into consideration in order for the method become for a commercial scale.

3.2.2. Fenton's Reaction Treatment

A physical-chemical method proposed by Steriti et al. [61] using the Fenton's reaction to disrupt the rigid cell wall of microalgae is considered to be a novel technique for microalgae lipid extraction. Fenton's treatment is an advanced oxidation process, which has a low degree of toxicity and typically being used for refractory humic acid degradation [62]. The method utilized hydrogen peroxide (H_2O_2) and Iron (II) sulphate ($FeSO_4$) to create the Fenton process.

According to Wu et al. (2010), the ferrous ions act as the catalyst reagent is capable to produce hydroxyl radicals (•OH) from H_2O_2. Then, the humic acid will be oxidized by hydroxyl radicals (proton abstraction) to form highly reactive organic radicals (R•). At this condition, the organic radicals can undergo oxidation and degradation.

Figure 6 illustrates the mechanism of the Fenton's reaction. The reaction mechanism is well described by the literatures [61, 62]. The reaction between the ferrous ion and H_2O_2 formed •OH as one of the products and attacked the microalgae cell wall (Fig.6a). This caused the breakage of the cell wall and lead to intracellular lipid released into the surrounding (Fig.6b). They are some possibilities that the •OH will also attach to the lipid to form lipid peroxide which is the undesired product (Fig.6c). Thus, to prevent from this happening, addition of alcohol such as ethanol into the solution can stop the Fenton's reaction and also the formation of lipid peroxide (Fig.6d). Finally, by adding solvents such as hexane can enhance the extraction efficiency of the lipid (Fig.6e).

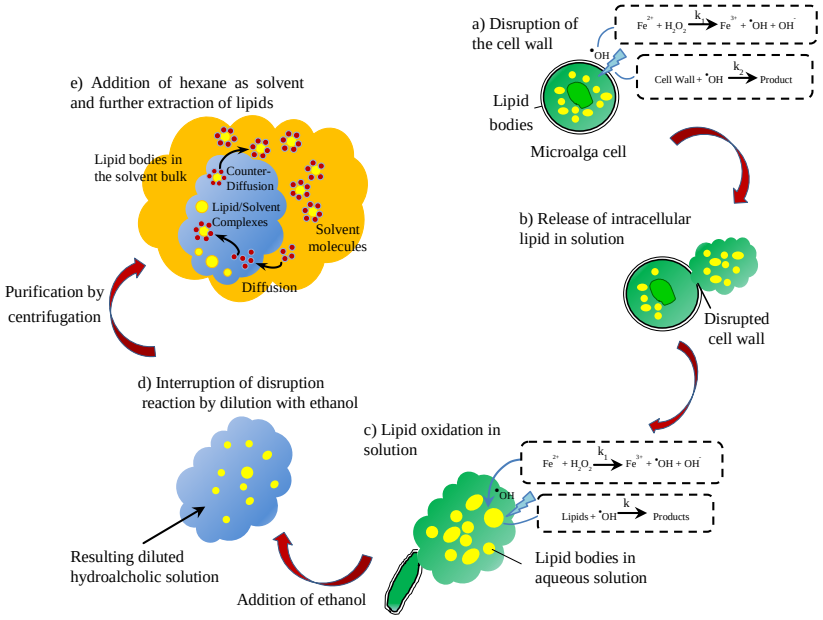

Fig. 6. The schematic diagram of the Fenton's reaction mechanism (Adapted and Modified from Steriti et al. [61]).

Table 2. Summary of novel cell disruption methods

Classifications of disruption method	Type of cell disruption method	Assisted extraction (after cell disruption)	Microalgae strain	Operating condition of extraction method	Lipid extraction (% w/w)	Ref.
Physical (Non-mechanical)	Electric Field lysis	-	*Chlorella vulgaris*	14.3 V/cm, 0.3A, 60mins, SS (Cathode), Ti/IrO_2 (Anode)	3.27	[59]
				30.5 V/cm, 0.3A, 60 mins, SS (Cathode), Ti/SnO_2 (Anode)	2.76	
				30.7 V/cm, 0.3A, 60 mins SS (Cathode), Ti (Anode)	2.65	
				26.3 V/cm, 0.3A, 60mins SS (Cathode), Ti/PbO_2 (Anode)	2.08	
Physical-Chemical (Non-mechanical)	Fenton's Reaction (Hydrogen Peroxide)	Solvent Extraction	*Chlorella vulgaris*	Fenton's treatment: 0.29 mol L^{-1} of H_2O_2, 4 mins Solvent extraction: $n\text{-}C_6H_{14}$: H_2O (3:2 ; v/v)	9.24	[61]
	Fenton's Reaction (Hydrogen Peroxide and Iron (II) Sulphate)			Fenton's treatment: 0.5 mol L^{-1} of H_2O_2, 0.024 mol L^{-1} of $FeSO_4$, 3 mins Solvent extraction: $n\text{-}C_6H_{14}$: H_2O (3:2 ; v/v)	17.37	

In the work of Steriti and co-workers [61], 9.24% w/w of *C. vulgaris* lipid can be extracted by using 0.29 mol L^{-1} of H_2O_2 for 4 minutes of treatment. Meanwhile, 17.37% lipid extracted was gained by using 0.5 mol L^{-1} of H_2O_2 and 0.024 mol L^{-1} of $FeSO_4$ for 3 minutes of treatment, which is 1.8 times greater than the Fenton treatment without the addition of $FeSO_4$. They also reported that, using H_2O_2 and $FeSO_4$ as the cell disruption method contributed FAMEs as high as 90%wt. Even though, 88% wt of FAMEs were attained from H_2O_2 treatment alone. Table 2 shows the summary of the novel cell disruption method. Both treatments are considered new methods for the extraction of lipid and valuable intracellular from microalgae crops.

4. Microalgae Lipid Extraction Based on Electroporation Concept

4.1. Theory of Electroporation Mechanism

Theoretically, the phenomenon which causes the cell wall to lyse is called electroporation. The exposure of microalgae cell wall to high electric fields for a certain period of time will lead the increasing of membrane permeability [19]. Electroporation can be divided in two categories they are reversible electroporation and irreversible electroporation. The term reversible electroporation can be defined as the cell membrane is still able to reseal back its membrane, while irreversible electroporation defines as the cell membrane unable to reseal back to its origin form.

The reversible electroporation concept is widely used in medical application to treat tumours (electrochemotherapy) by injecting the drugs into the patient's cell target. The cell is electrified with the proper strength in a short period just to allow the drugs go inside the cell and attack the tumour cell and then cell membrane will reseal back to its original form [63]. Meanwhile, irreversible electroporation concept is widely applied in food industries for microbial inactivation [64, 65], in engineering for lipid extraction [12, 13], in biotechnology for pigments, protein and carbohydrates extraction [66-68] and in wastewater treatment to kill pathogens and bacterial [12, 18, 69, 70].

Figure 7 shows the process mechanism of irreversible and reversible electroporation of a microalga cell membrane. In Fig.7a, two electrodes were placed between a microalga cell was in a static condition if no electric field, E applied to the cell, by means the cell was at zero potential. If the electric field was then introduced (lower than its critical strength, E_c) to the microalga cell, it will start to distract the polar bonds that hold together the molecules which formed the microalga cell wall (Fig.7b). By increasing the electric fields strength above the critical strength of the cell membrane, it will overcome the forces that bonds the bilayer together (Fig.7c), since the bonds held together by non-covalent interaction [71] resulting in the formation of pores (holes in the membrane).

According to Zimmermann [72] the accumulation of the free charges at both sides of the membrane will cause the membrane to compress and a mechanical breakdown. The high exposure of electric fields will cause in the enlargement

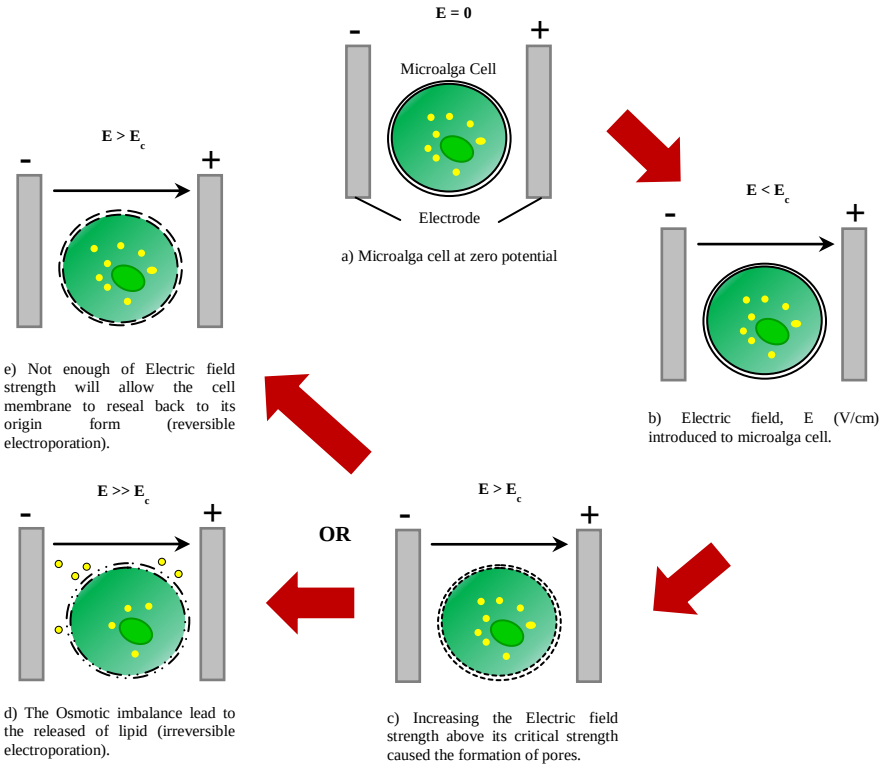

Fig. 7. The electroporation mechanism of microalga cell membrane.

of pore formation and increase the number of pores. This can explain due to the electrostatic interaction between the cell membrane (electropermeabilization) will increase if the amount of electric field applied to the cell is increased. Therefore, this result cause in the enlargement of pores in the cell wall [15, 73]. Thus, the cell will experience an osmotic imbalance due to the pressure difference inside and outside the membrane.

Once the pores formed, the intracellular (i.e. lipids) will be drained out from the cell membrane (Fig.7d) and this called as an irreversible electroporation mechanism [64, 71]. At a certain point, they were possibilities that the bilayer molecules capable to reseal back to its original structure condition due to the strong interaction among all the molecules to pull back together (Fig.7e). The mechanism, called as the reversible electroporation [74].

4.2. The Factors Affect the Electroporation Mechanism

Electric field strength can be defined as the amount strength (voltage supplied, V) over distance (cm) to lyse the cell membrane of a microorganism. It is very obvious that the amount of field strength applied is directly proportional to the treatment efficiency [75]. It was also reported that, increasing field strength provides better efficiency rather than increasing the pulse duration. Amplitude value is the maximum peak of electric field strength applied in PEF. In food application, the elevation of pulsed width number is correlated with treatment time that being supplied will enhance the treatment duration as a purpose to increase the inactivation of microbe in the foods [76].

The type of electrode materials is also crucial for attaining a good efficiency of the electroporation mechanism. Several electrode materials such as nickel, silver or gold gives homogeneous electric field distribution [77]. Based on reported literatures so far, most researchers had used electrode made from stainless steel material as a part of their chamber design [12, 13, 15, 19]. This is because stainless steel electrode is cheaper, highly available in the market and also a good electrical conductivity. However, some researchers had used electrode made from gold and silver chloride [14] metal oxide such as titanium, titanium insulated with Iridium (IV) Oxide and Titanium insulated with Tin (IV) Oxide [59] as suggested by Bushnell et al. [78].

Cell membrane treatment duration is dependable on the characterization of microalgae such as size and shape. More energy is required to break the cell walls of microalgae compare to microalgae that has no cell wall (i.e. *Dunaliella Salina*) [14].

5. Pulsed Electric Fields Technology

5.1. Pulsed Electric Fields System

Pulsed Electric Fields (PEF) technology is a non-thermal method that is extensively used in food processing applications. This equipment is commonly used in the process of inactivation of microbes in food, so that it can be well preserved and safely consumed by human [79]. PEF technology is considered to be a promising method for microalgae lipid extraction due to economic in energy consumption [18], highly potential for scale up [15, 80-82] and economic in operation costs [18, 65] compared to conventional extraction methods.

Generally, PEF system can be divided with three basic components, a control system to monitor the parameters for

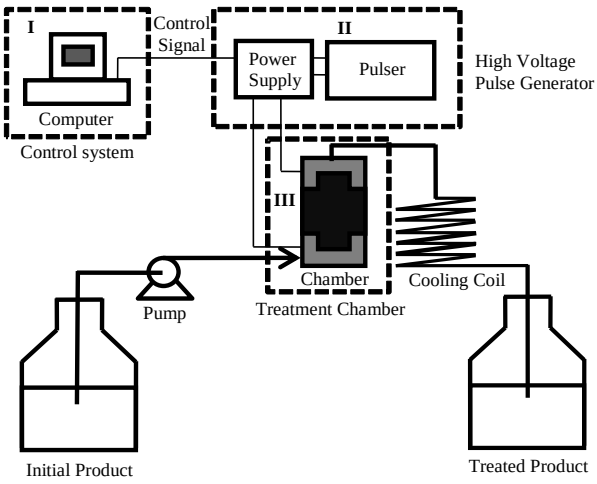

Fig. 8. Operation of PEFs (Adapted and modified from Ortega-Rivas, [64]).

the treatment process (I), a high voltage pulse generator that is to supply voltage (II) and a treatment chamber which is used to treat the target sample (III) illustrated in Fig.8. The voltage can be high as 45 kV with microsecond (µm) to milliseconds (ms) of pulse duration. In fact, the types of pulse also can be selected such as exponential decay and square wave. The design of a treatment chamber may also be different. Meanwhile, the control system normally is fabricated together with a power supply and a pulser.

5.2. PEF Treatment Chamber Design

5.2.1. Parallel Plate Treatment Chamber

The design of parallel plate static is the simplest chamber design used for microalgae lipid extraction treatment in batch system and suitable for laboratory scale. The plate electrodes are placed in a parallel position so that uniform field strength can be achieved. However, the lack of the design is the distances between the electrodes need to be smaller than the surface dimension of the electrode [76]. Since, one of the parameters influenced the strength of the electric field is the gap between the electrodes.

A laboratory work using PEF-modified in a 4 mL UV cuvette designed by Zbinden [19] is shown in Fig.9. Square shape of stainless steel plate electrodes with a surface area of 64 mm^2 each and gap of 10 mm were placed between the *A. falcatus*. The height, length and width of the cuvette were 44.6 mm, 10.4 mm and 10.4 mm respectively.

Meanwhile, Foltz [14] had designed the simplest chamber for cell lysis observation using silver chloride electrodes glued on view slides of glass microscope. The electrode gap was 1 mm with a sample depth of 0.25 mm (Fig.10).

A continuous flow of parallel chamber was designed by Goettel et al. [13] using stainless steel electrodes (diameter of 60 mm) with a treatment volume of 2 ml (47 mm length, 11 mm width) and electrodes spacing of 4 mm shown in Fig.11.

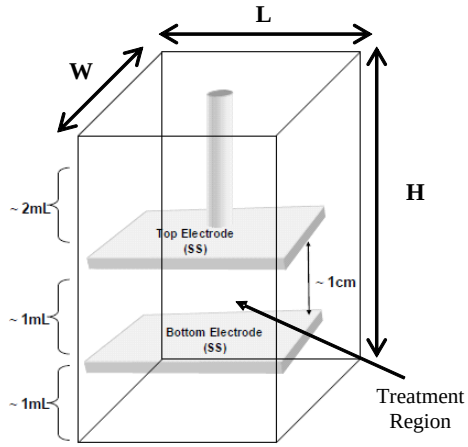

Fig. 9. Parallel plate static treatment chamber in modified UV cuvette designed by Zbinden [19].

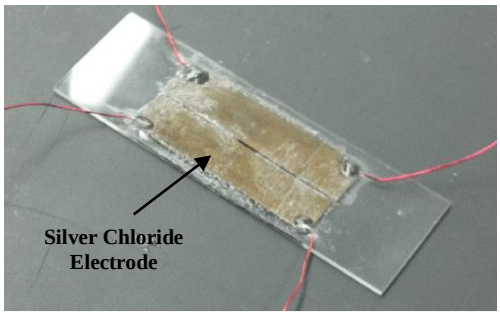

Fig. 10. Parallel plate static treatment chamber designed by Foltz (2012).

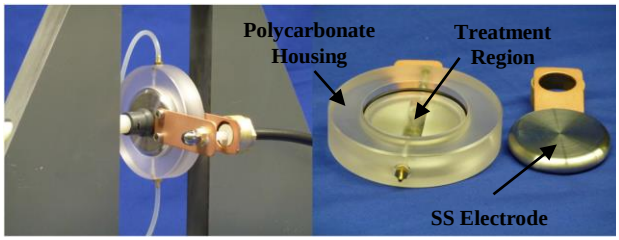

Fig. 11. Parallel plate treatment chamber designed by Goettel et al. [13].

5.2.2. Co-Field Treatment Chamber

The co-field treatment chamber design is mainly used in continuous system and much suitable for large scale. The advantage of using this type of design is that the supplied voltage value is approximately the same with the voltage across the treatment region [79]. Flisar and co-workers had designed a prototype of a co-field treatment chamber using stainless steel electrodes, insulated with polytetrafluoroethylene and has a gap electrode of 15 mm [12]. The details dimension of the chamber design is shown in Fig.12. Aside from that, a commercial PEF chamber designed by Kempeks et al. [15] which was compatible for a wide range of flow production from 10 to 100,000 L/hr. The chamber has four treatment cells and a pipe diameter approximately 1.5 cm (Fig.13). Nevertheless, it is crucial to

take note that if using this type of chamber design, the pipe diameter is directly proportional to the voltage supply. According to Kempeks et al. [15] increasing the pipe diameter may also require a high pulse voltage to achieve constant field strength.

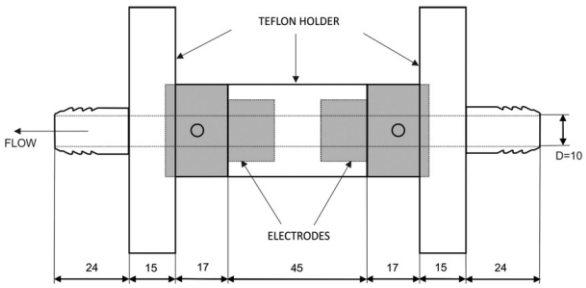

Fig. 12. A co-field treatment chamber designed by Flisar et al. [12].

Fig. 13. A co-field treatment chamber designed by Kempkes et al. [15].

6. PEF Treatment as the Method for Microalgae Lipid Extraction

The extraction via PEF has been used directly or as a pre-treatment prior to solvent extraction method to break the microalgae cell wall. A recent work by Lia et al. [17] on the effect of PEF towards lipid recovery of *Scendesmus spp*.. They used the Focused-Pulsed (FP) technology, which an adaptation of PEF technology as the prelude for the microalga treatment. The commercial technology was provided by OpenCEL [83]. Unlike, Sheng et al. [21] they had conducted two passes treatment for the microalga cell disruption. First treatment the microalga was exposed with intensity of 17.9 kWh/m^3 and was kept overnight before exposing to the second treatment with intensity of 33.7 kWh/m^3. Four solvents system was used in their work for lipid extraction enhancement. They found that, Floch method associated with FP extraction show the highest extraction performance which was about 34% wt of crude lipid to biomass ratio followed by the Bligh and Dryer method, hexane and isopropanol. The study had observed that, 97% of the microalga cells were electroporated (based on stained cells for inactive cells) by the FP treatment prior to solvent extraction.

Flisar et al. [12] had demonstrated a study on the extraction of lipid from *C. vulgaris* and the inactivation of *E.coli*. 22% wt of total lipids can be extracted using 21 × 100 μs pulses, repetition frequency of 10 Hz and 2.7 kV/cm field strength. Meanwhile, Eing et al. [18] combined PEF and solvent extraction method and gained 22% wt of lipid from *A. protothecodies* using higher strength of 35 kV/cm. This can be explained that different species of microalgae may respond to different field strength.

Goettel and co-works have also conducted a study on PEF extraction of intracellular on *A. protothecodies* [13]. The work was conducted in continuous flow and the pulse chamber system was manufactured by the Institute of Pulsed Power and Microwave Technology (Karlsruhe Institute Technology, Germany). Based on their results, the extraction efficiency increased after microalgae was treated with PEF with the field strength of 23 kV/cm to 42 kV/cm but no lipids were detected. They stated that only soluble cell from the microalgae drained out during the PEF treatment and lipids still remained inside of the cell membrane. Moreover, it was found that the microalgae concentration does not affect the intracellular extraction performance. However, using the PEF as a pre-treatment and ethanol as solvent extraction in the final stage, gave a better result on *A. protothecodies* lipid extraction [18].

Zbinden et al. [20] had also used the PEF as a pre-treatment to break the cell wall of *A. falcatus* a needle like shape of microalga and had also modified the Bligh and Dryer method extraction to extract the lipid content. The high voltage pulsed generator was designed by ARC Technology LLC, Whitewater, KS. The PEF treatment found to be capable of increasing the lipid extraction efficiency to 90% at field strengths of 45kV/cm and pulse duration of 100 ns. Greener solvent was used in their studies, replacing chloroform to ethyl acetate. Approximately about 42 J/mL of energy input was consumed during the PEF treatment and was conducted in laboratory scale.

In the work of Foltz [14], using silver/silver chloride as the electrode to lyse *Chlamydomonas reinhardtii* and *D. salina*, while stainless steel electrode used to lyse *C. vulgaris*. He had claimed that, the electric field magnitude only helps the enlargement of the pore mechanism of cell wall and not disrupt the cell wall. He also observed that, at 150 kV/cm cell *C. reinhardtii* can be electroporated in 33.3 second. However, *D. salina* cell can be lysed at 1.6 kV/cm with repetitive lower amplitude pulses and at 8.0 kV/cm with a single pulse of suitable amplitude. Moreover, at 4.0 kV/cm *C. vulgaris* cell wall can be lysed with repetitive lower amplitude. Compared to the other two microalgae strains, *D. salina* required less energy or lower field strength due to its cell structure that has no cell wall.

Sheng et al. [21] used FP followed by solvent extraction method using isopropanol to extract the intracellular lipids from cyanobacteria *Synechocystis PCC 6803*. Similar to PEF, the FP also suitable to be used for denitrification and methanogensis in industrial scale. By utilizing the low solvent/wet biomass ratio of 5, they had managed to achieve lipid recovery up to 75% which was calculated based on the FAMEs content. The intensity of 17.9 kWh/m^3 and outflow temperature of 36˚C was found to be the optimum value for treatment above than 500 μs.

Table 3. Summary of microalgae lipid extraction via PEF technology

Reference (s)		[12]	[13]	[14]	[15, 16]	[17]	[18]	[19, 20]	[21]
Purpose of Study		Lipid extraction	Intracellular and lipid extraction	Cell lyse observation	Lipid extraction	Lipid extraction	Lipid extraction	Lipid extraction	Lipid extraction
Microalga Strain (s)		*Chlorella vulgaris*	*Auxenochlorella protothecodies*	*Chlamydomonas reinhardtii, Dunaliella salina & Chlorella vulgaris*	*Isocrysis sp.*	*Scenedesmus spp.*	*Auxenochlorella protothecoides*	*Ankistrodesmus falcatus*	*Synechocystis PCC 6803* (cyanobacteria)
Size/Shape		Diameter: 2.6-5 µm/Spherical	Diameter:5-8µm/Spherical	*a:* 10 µm /Spherical; *b:* 9-11 µm /rod to ovoid shaped; *c:* 2-10 µm /Spherical	Diameter:3-5µm/Spherical to pear shaped	12 or more cells; length 12.5 µm, width: 5 µm/Oval to box-shaped	Diameter:5-8µm/Spherical	Diameter:3 µm; length: 40 µm/ Needle-like	Spherical
Lipids content by % of dry weight		14 - 40	20 - 25	*a:* NA; *b:*14 - 20; *c:* 14 - 40	NA	19.6 - 21.1	20 - 25	40	NA
Batch or Continuous Treatment System		Continuous	Continuous	Batch	Continuous	Batch	Continuous	Batch	Batch
Volume of Microalgae Cultivation		Closed Photobioreactor (50 L)	Closed photobioreactor (26 L)	NA	One embodiment of a PEF treatment (rectangular)	Closed photobioreactor (40 L)	Closed photobioreactor (26 L)	Chemostat (3 L)	Transparent Carboy (25 L)
Work Scale		Laboratory	Laboratory	Laboratory	Commercial	Laboratory	Laboratory	Laboratory	Laboratory
Volume of Microalgae		-	-	*a*: 1.25 µL; *b*: 312.5 nL; *c*: 10 µL	-	40 L	-	-	-
Biomass Concentration		-	36 - 167 g/kg	-	-	≈ 15 g	100 g/L.	1.9 g/L	≈ 0.3 g/L
Fed Flowrates, F		43.2 L/hr	0.36 L/hr	-	10 - 100,000 L/hr	-	0.36 L/hr	-	-
Extraction Method	**Pre-treatment**	PEF	PEF	PEF	PEF	FP	PEF	PEF	FP
	Solvent Extraction	-	-	-	-	B&D, Floch, Hexane, Isopropanol	Ethanol	Modified B&D	Isopropanol

PEF Chamber Design	Co-field flow chamber	Paired in parallel with polycarbonate housing	Cubic millimeter chamber	Co-field flow chamber	Co-field flow chamber	Paired in parallel with polycarbonate housing	Modified UV cuvette	Co-field flow chamber
Electric Field Strength, *E*	2.7 kV/cm	23 k - 43 kV/cm	*a*: 0.15 kV/cm; *b*: 1.6 kV/cm; *c:* 4.0 kV/cm	20 k - 30 kV/cm	1st pass treatment: 30.6 kWh/m^3; 2nd pass treatment: 33.7 kWh/m^3	35 kV/cm	45 kV/cm	>35 kWh/m^3
Pulse shape	Square wave	Square wave	*a*: Square wave; *b* & *c*: Exponential Decay	Square wave	Square wave	Square wave	Exponential decay	Square wave
Pulse Frequency, *f*	10 Hz	1.0 - 5.5 Hz	*a*: 14.92 Hz *b*: 0.2 Hz *c*: 3.0 hz	10 – 50 kHz	NA	1.0 - 5.5 Hz	210 Hz	NA
Number of Pulses, *N*	21	NA	*a*: 476 *b*: 5 *c*: 60	NA	NA	NA	21	NA
Pulse duration, *t*	100 µs	1 µs	*a*: 33.3s; *b*: 50s; *c*: 20s	1 µ - 10 µs	> 500 µs	1 µs	100 ms	> 500 µs
Type of electrode material	Stainless Steel	Stainless Steel	*a* & *b*: Silver/ Silver Chloride *c*: Stainless Steel	NA	NA	Stainless Steel	Stainless Steel	NA
Distance between electrodes, *d*	15 mm	4 mm	*a*: 5mm; *b*: 5mm; *c*: 0.25mm	0.8 cm	NA	4 mm	10 mm	NA
PEF specific treatment energy	14.4 kJ/L	52 - 211 kJ/kg	*a*: 19.47 mJ *b*: 528 mJ *c*: 2.475 J	≈ 10 k - 300 kJ/L	NA	200 kJ/kg_{sus}	≈ 42 J/L	NA
Extracted Lipid	22 % wt	NA	NA	NA	B&D: ≈ 23% wt ; Floch: ≈ 34% wt; Hexane: ≈ 10% wt; Isopropanol: ≈ 6% wt	22 % wt	6.1 mg/L	25 to 75% wt [d]

Notes:
a referred to *Chlamydomonas reinhardtii*.
b referred to *Dunaliella salina*.
c referred to *Chlorella vulgaris*.
d Extracted lipid as Fatty Acids Methyl Ester (FAME) in % wt.

B&D referred to Bligh & Dyer Method.
NA- Not available (Data not mentioned by the authors).

A United State patent published by Kempkes et al. [15] had designed a single equipment using PEF to extract lipids from *Isocrysis sp*. The technology was provided by Diversified Technologies, Inc. [84] with the efficiency of power conversion up to 90%. The design was a continuous system and suitable with input flow rates of 10 to 100,000 litres per hour, hence suitable for large scale application. In addition, the pulse only required 20 to 30 kV/cm for 1 to 10 microseconds to lyse the microalgae cell wall. Table 3 shows the summarized table of the lipid extraction from microalgae via PEF Technology. It is hardly to conclude that, which studies conducted were the most efficient due to each work was implemented in different operating condition. However, PEF treatment alone or associated with solvent extraction afterwards is proven to enhance the extraction efficiency of lipid from microalgae.

7. The advantages and limitations of PEF

Table 4 shows the lists of advantages and limitations of using PEF as an extraction method for microalga lipid extraction. By using PEF extraction alone or without the additional chemicals such as solvents, it has been proven to increase the lipid extraction as reported from literatures [12, 17-21]. PEF does not affect the quality of the products (i.e. FAMEs composition) as proven by Lia et al. [17]. No need of drying process or removal of water since the technology is suitable for wet or dry microalgae [13, 81]. The technology has high scalability and already been conducted in commercial scale [15, 81, 83, 84]. No heat is involved due to its non-thermal behaviour adaptation from food industries application [64], economical energy consumption [18] and less time consuming [13, 20]. In addition, using PEF as pre-treatment prior to solvent extraction also has proven to increase the lipid extraction efficiency and also decrease the usage of solvent, hence reduce the extraction cost [17].

Table 4. Advantages and limitation of PEF technology

PEF as the Method for Microalgae Lipid Extraction	
Advantages	PEF as a single step extraction: 1. Does not involve dewatering or drying process, thus reduce the operational cost. 2. No addition of chemicals, therefore, reduces the operational cost. 3. No heating (non-thermal equipment), hence, use less energy. 4. Suitable to treat wet or dry microalgae. 5. Does not affect the quality of the product. 6. Less time consuming (treatment time from microsecond to second). 7. Highly scalability. PEF as a pre-treatment extraction: 1. Great combination with solvent extraction and proven to increase the lipid extraction efficiency. 2. With PEF treatment the amount of solvent can be reduced.
Limitations	1. Dielectric breakdown can be affected by the existing of air bubbles in the treatment chamber and causing the PEF treatment to become less uniformity. 2. The cell membranes can be reversible or irreversible during electroporation mechanism based on the electric field strength being applied. 3. The efficiency of the PEF highly dependable with the amount of electric field strength and electrode gap.

However, according to Góngora-Nieto et al. [85] the present of air bubbles in PEF chamber may lead on the decreasing uniformity of the electric field strength due to dielectric breakdown. The electric field strength may also affect the destruction of microorganism cell walls and at this point the cell membranes can be in a reversible condition where the cell walls not disrupted or at the irreversible condition where the cell walls damaged [86]. Apart from that, PEF design is very important because it can also affect the efficiency of the equipment. For large scale production, the production flow rate is dependable of the PEF pipe diameter, thus the higher field intensity is needed for maintaining the same amount of field strength distribution [16].

8. Conclusion

Microalgae crops as renewable energy specifically for biodiesel production has been extensively studied due to its high lipid content, rapid growth, environmentally friendly and capable of reducing CO_2 emission. In the meantime, the extraction technology is one of the major challenges in biodiesel production for obtaining a high yield from the microalgae. Conventionally, microalgae lipid can be extracted using several methods such as microwave assisted extraction, solvent extraction, supercritical fluid extraction and enzymatic method, however, may face some drawbacks such as difficult to scale up, high toxicity, time consuming and expensive. The study revealed the potential of PEF technology for microalgae lipid extraction. PEF treatment technology is found to be a promising technology for microalgae lipid extraction due to its advantages such as non-thermal behaviour, less time consuming, does not require any additional chemicals and high scalability for massive production. Moreover, combining PEF and solvent extraction method has proven to improve the efficiency of microalgae lipid extraction. PEF not only been used for lipid extraction purpose but also for protein, chlorophyll and carbohydrate extraction.

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