

Review Paper on Cell Membrane Electroporation of Microalgae using Electric Field Treatment Method for Microalgae Lipid Extraction

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Abstract. The paper reviews the recent studies on the lipid extraction of microalgae that mainly highlighted on the cell disruption method using variety of microalgae species. Selection of cell disruption method and devices are crucial in order to achieve the highest extraction percentage of lipid and other valuable intracellular (proteins, carotenoids and chlorophylls) from microalgae cell. Pulsed electric field (PEF) and electrochemical lysis methods were found to be potential for enhancing the extraction efficiencies either conducted in single step extraction or used as pre-treatment followed by conventional extraction method. The PEF technology capable to extract lipid as high as 75%. While, electrochemical lysis treatment capable to extract lipid approximately 93% using Stainless Steel (SS) and Ti/IrO₂ as the cathode and anode electrode respectively. PEF technology and electrochemical lysis are still considered to be a new method for microalgae lipid extraction and further investigation can still be done for better improvement of the system.

1. Introduction

The major concern of fossil fuel depletion has stressed out the importance of seeking an alternative renewable energy that fulfilled the criteria such as economic, sustainable and environmental friendly. The third generation of biofuel, which is biodiesel from microalgae had demonstrated to be the most potential biomass feedstock in replacing the other conventional crops like maize, jatropha and palm oil [1-3]. In comparison with the conventional crops based biodiesel, microalgae provide several benefits such as high growth rate [4; 5], high lipid content [6] and reduce in land requirements [7]. In addition, the issues regarding food versus fuel are less affected for microalgae crops, but yet still being used as an additional supplement in the human diet and animal nutrition such as *Spirulina* and *Chlorella* [7].

Fresh water microalga *Chlorella* species has been clearly stated to be a most favorable strain due to its high lipid content which rich with saturated fatty acids and suitable for biodiesel conversion [6; 8; 9]. Biodiesel yields are much more related to the percentage of lipid extracted from the microalgae cell. Hence, the selection of extraction methods and devices are totally dependable on the extraction efficiencies of lipid extracted from the microalgae biomass. Microalgae lipid can be extracted by traditional methods either via chemical, physical, enzymatic or a combination of the techniques. The



methods included solvent extraction, supercritical fluid, microwave irradiation, osmotic shock, and homogenizer. Although these methods are still being used for lipid extraction, there are several drawbacks of using the traditional lipid extraction methods such as time consuming, energy intensive, potentially harmful and difficult to scale up [10].

Presently, microalgae lipid extraction using the concept of cell membrane electroporation or electropermeabilization has gained interest lately. Pulsed electric fields (PEF) and electrochemical lysis methods had demonstrated to be able to increase the lipid extraction efficiency either used as pre-treatment or alone and potential for large scale application [11-20]. Besides, the methods have the advantages of eliminating dewatering process since the methods are applicable for direct wet biomass. The methods consume less energy and no additional chemicals required. Industrial scale application using PEF for microalgae lipid extraction [21] and algae wastewater treatment [22] had already been demonstrated.

To the best of our knowledge, the cell disruption related to the electroporation concept for microalgae lipid extraction has not yet been practiced in Malaysia country. The methods are relatively new for microalgae lipid extraction and only few literatures had been published so far. This paper focused on the potential application of PEF and electrochemical lysis treatment as the method to extract microalgae lipid for wet or dry biomass. The paper also includes the theory of electroporation mechanism. The methods can be an alternative approach for lipid extraction in biodiesel production specifically for algae based crop.

2. A cell membrane electroporation theory

In the middle of 1900, the electroporation concept had already been used in medical application. The concept then applied in food technology and processing to kill microbes and pathogens [23]. The concept is kindly unique, therefore, has attracted researchers to use it in biotechnology and engineering application specifically to extraction lipid and other valuable intracellular (e.g. Pigments and protein) from microalgae. Electroporation is a phenomenon which the cell wall exposed to high electric field strength in a certain period. During the electric field exposures the cell membrane is temporarily destabilized. Increasing the electric field intensity will cause the cell membrane to experience either reversible or irreversible electroporation [24]. Reversible electroporation is the condition which the cell membrane capable to recover or reseal back its membrane. While, irreversible electroporation is the cell membrane permanently damage and unable to recover its original form.

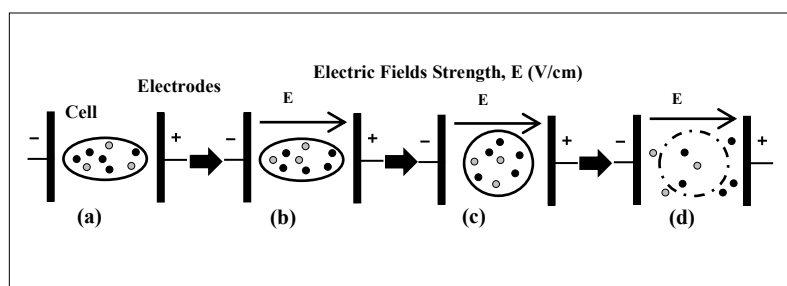


Figure 1. Electroporation Mechanism of a cell membrane: (a) At zero potential, (b) Osmotic imbalance, (c) Swelling, (d) Membrane ruptures. Modified from Ortega-Rivas [23].

Chemotherapy method for cancer treatment is using reversible electroporation to allow the injected drugs to attack the cell cancer after the cell membrane reseal back its initial form [25]. But from the engineering side of view, irreversible electroporation is preferable for the extraction of valuable components from the cell such as lipid and proteins. Figure 1 shows the illustration of the cell membrane electroporation mechanism. By placing a cell between two electrodes without introducing the electricity or at zero potential gives the cell in a static condition shown in figure 1a. The exposure

of electric fields induced the cell causing an osmotic imbalance and swelling of the cell. At this moment, the cell membrane molecules that hold by the polar bonds were distracted by the electric field induction (figure 1b and figure 1c). In this state also, the formation of pores among the cell wall of membrane begin and the reversible electroporation may occur. The increasing of electric field strength will cause the cell membrane to break due to amount of pores increase. By maintaining the electric field strength above its threshold value or its critical strength, the cell wall will be electroporated [26]. The valuable components such as lipid, carotenoids, chlorophylls and protein will be released from the cell that shown in figure 1d. At this rate, the membrane will no longer be able to recover from its original form and this stated as irreversible electroporation [23]. The effectiveness of the membrane to be electroporated affected by several factors, for instance the size of the microorganism. Microorganisms that have smaller size required higher electric field strength for the cell wall to be broken down [27]. This is likely due to the thermal and mechanical fluctuation condition of the cell membrane [28].

3. PEF as the method for lipid extraction

Pulsed electric field (PEF) technology is a non-thermal method that is widely used in medical application for cancer treatment [25], in biotechnology for DNA transfer [29] and in food industries for microbial inactivation [23]. Lipid extraction from microalgae using PEF technology has received notable attention in the past few years. Generally, PEF system includes pulse generator, treatment chamber and control system. The technology is well known to be a promising method for microalgae lipid extraction due to low energy consumption, potential to be scaled up and economic [30]. The recent studies on microalgae lipid extraction in the application of PEF is discussed in this section. Table 1 shows the present studies done by several authors using the PEF as a single step extraction or as a pre-treatment followed by conventional method (i.e. Solvent extraction). From the table of summary, variety of microalgae species such as *Chlorella vulgaris*, *Spirulina*, *Nannochloropsis sp.*, *Auxenochlorella protothecoides*, *Ankistrodesmus falcatus*, *Isocrysis sp.* and *Synechocystis PCC 6803* cyanobacteria a blue-green algae were used in their studies.

Flisar et al. [11] had studied the lipid extraction from *C. vulgaris* using PEF in a continuous flow system. Lipid content in microalga *C. vulgaris* is about 50 to 58% dry weight biomass. They fabricated the PEF treatment chamber using stainless steel as the electrodes and the housing act as an insulator was made from polytetrafluoroethylene material. The chamber designed with a co-field shape and the gap between the electrodes was fixed to 15 mm. The study found that, approximately 50% (0.43g of lipid/g of dry biomass) of lipid was extracted using electric field strength of 2.7 kV/cm for 21 pulses of 100 μ s. According to Luengo et al. [13] microalga *C. vulgaris* that being exposed to 10 kV/cm electric field strength and 50 pulses for 3 μ s was found to be at reversible electroporation state. But, increasing the field strength to 20 to 25 kV/cm and utilized lower pulses of 5 for 3 μ s will cause the cell membrane at irreversible electroporation state. The electrodes distance used in their work was 2.5 mm which smaller than the distance used by Flisar and co-workers [11]. Foltz [18] had also conducted a study on *C. vulgaris* using PEF for cells lyse observation using smaller electrode distance of 0.25 mm. In his experiment, the cell can be lysed using the treatment condition of 4 kV/cm electric field strength and 60 pulses for 20 seconds. Based on the results reported from the three literatures, lower electric field strength and smaller distance between the electrodes can be used for the irreversible membrane permeabilization to occur. Longer time is required to lyse the cell membrane with lower electric field strength and larger space between the electrodes. Moreover, the size of microalga *C. vulgaris* is 2 μ to 10 μ m which is very small thus, required higher voltage to break the cell wall.

Eing and co-workers had demonstrated that the percentage extracted lipid from *A. protothecoides* can be enhanced associated with solvent extraction after treating with PEF [15]. They constructed the PEF treatment chamber from polycarbonate and stainless steel electrodes with 4 mm electrode gap. Then the target sample was exposed to 35 kV/cm electric field strength for 1 μ s duration but no lipid was detected. The result obtained had disagreed with previous literature that used lower field strength

to extract lipid [11]. The best assumption that can be described, was the behavior of the cell wall of different microalgae species may permeable to certain water-soluble or ions but not specific to the lipid droplet [15].

Table 1. Summary of PEF application in microalgae.

Microalgae strain	Purpose of Study	Scale	Cell Disruption /Solvent Extraction	Lipid Extracted (% wt)	Electric Field Strength, E (kV/cm)	Reference
<i>Chlorella vulgaris</i>	Extraction of lipid	Lab	PEF	22	2.7	[11]
<i>Spirulina</i>	Inactivation of microalgae	Lab	PEF	NA	33.3-66.7	[12]
<i>Chlorella vulgaris</i>	Extraction of pigments	Lab	PEF	NA	20-25	[13]
<i>Nanno-chloropsis sp.</i>	Extraction of intracellular	Lab	PEF	NA	20	[14]
<i>Auxenochlorella protothecoides</i>	Extraction of lipid	Lab	PEF/ C ₂ H ₅ OH	22	35	[15]
<i>Auxenochlorella protothecoides</i>	Extraction of intracellular	Lab	PEF	NA	23-43	[16]
<i>Ankistrodesmus falcatus</i>	Extraction of lipid	Lab	PEF/H ₂ O, C ₄ H ₈ O and CH ₃ OH	6.1 mg/L	45	[17]
<i>Dunaliella salina</i>	Observation of cells lyse	Lab	PEF	NA	1.6	[18]
<i>Chlorella vulgaris</i>	Observation of cells lyse	Lab	PEF	NA	4	[18]
<i>Synechocystis PCC 6803^a</i>	Extraction of lipid	Lab	FP ^b / C ₃ H ₇ OH	25-75 ^c	>35 ^d	[19]
<i>Isocrysis sp.</i>	Extraction of lipid	Industrial	PEF	NA	20-30	[20]

^a Cyanobacteria strain.

^b Focused Pulsed (FP) adaptation of PEF technology.

^c Extracted lipid as Fatty Acids Methyl Ester (FAME) in % wt.

^d Treatment intensity unit in kWh/m³.

NA (Data not available).

Besides lipid, other valuable components from *A. protothecoides* such as organic carbon, carbohydrates and proteins can be also extracted using PEF treatment. The work implemented by Goettel et al. [16] on the extraction of intracellular valuable from *A. protothecoides* and using the similar PEF treatment chamber with Eing and co-workers. The study used electric field strength of 23 to 43 kV/cm to disintegrate the cell wall to allow the intracellular to be drained out. It was observed that no lipid droplet is released from the cell which supported the result gained from Eing and co-

workers. However, it was found that 1 MJ/kg_{dw} algae is required to lyse the cell wall of *A. protothecoides* for 100g dry weight over kg of suspension.

Qin et al. [12] had investigated the inactivation of *Spirulina* using the PEF method. Inactivation can be defined as the cell wall of microalga was damaged by the induction of electric field strength. The results indicated that the *Spirulina* can be lysed at field strengths of 33.3 kV/cm and impulses number of 100 to 500. The increasing of field intensity to 66.7 kV/cm will decrease the number of impulse to 50. Thus, the number of impulse is inversely proportional to electric field strength. Microalga *Spirulina* has larger size compared than that of *C. vulgaris*, *Nannochloropsis sp.* and *Isocrysis sp.* which is 0.5 mm in length. Different microalgae species and sizes can affect the performance of PEF to disintegrate the cell wall. Antezana-Zbinden et al. [17] had conducted a study on *A. falcatus* lipid extraction using PEF as the pre-treatment to disrupt the cell wall and followed by the solvent extraction. The solvents used were ethyl acetate, methanol and water. Ethyl acetate was selected instead of chloroform solvent due to less harmful to the environment. Using PEF prior to solvent extraction increased the lipid extraction. Approximately 90% of the cell algae had been electroporated at the condition of 45 kV/cm field strength in 100 ms. The PEF caused the disarrangement of the cell wall membrane molecules and using high field intensity attributed to irreversible electroporation. Then, allowing the solvent molecules to extract the lipid droplet and thus, this has enhanced the percentage of lipid recovery.

Certain of microalgae can be lysed easily with lower electric field strength as 1.6 kV/cm such as microalga *D. salina* [18]. This can be explained that some of the microalgae do not have a cell wall. Other than green microalgae, cyanobacteria (a blue - green algae) also been used for the test subject. Sheng et al. [19] reported that using PEF prior to solvent extraction increased the efficiency of lipid extraction from the dry or wet biomass. The study indicates that 87% of the cell being electroporated using PEF at treatment intensity of 35 kWh/m³. It was found out that, it was no efficient to use isopropanol without the PEF treatment. But, with PEF treatment 25 to 75% of lipid recovery can be achieved using solvent to wet biomass ratio of 5. Although, pilot scale for microalgae lipid extraction using PEF has not yet been reported, the PEF application already had been patented in industrial scale. United State patent published by Kempkes and co-workers had designed a single equipment using the PEF concept to extract lipids from *Isocrysis sp.* [20]. The technology was provided by Diversified Technologies, Inc with the efficiency of power conversion up to 90%. The design was operated in continuous system and suitable with input flow rates of 10 to 100,000 liter per hour, hence suitable for large scale application. Additionally, the pulse only required 20 to 30 kV/cm for 1 to 10 microseconds to lyse the microalgae cell wall. In overall, PEF consumed less energy which is 4.6 times lower compared to conventional drying method for lipid extraction [15; 19].

4. Electrolysis as the method for microalgae lipid extraction

The electrolysis method utilizes electrically charged probes called an anode and a cathode electrode, which been submerged in salty water to separate the oxygen and hydrogen atoms. The technique uses two different electrodes to induce an electric field due to the different conductivities of the electrode materials supplied by lower voltage (typically from 10 to 30V). In comparison with PEF method that required a higher voltage range from 1 kV to 60 kV in a short period of time (nanosecond to microsecond). Lower voltage requires longer time of treatment and vice versa.

Electrolysis method is basically being used during the harvesting process in microalgae production to enhance the biomass concentration [31; 32]. A recent study conducted by Daghrir and co-workers using electric field formed by electrochemical method to lyse the cell wall and extract intracellular components of microalga *C. vulgaris* [33]. They used the electrochemical treatment terms as the method to extract lipid from the microalga in their studies. Treatment chamber was fabricated using plexiglass material and using 1 liter of microalga sample for the test subject. Both cathode and anode electrode plates were placed parallel with 1.0 cm distance and submerged into the electroporation chamber. Stainless steel was fixed at cathode mode while 4 types of electrodes were used at anode mode (table 2). Since, the voltage used was lower compared to PEF, thus longer treatment time is

required. By fixing the treatment time at 60 minutes, Ti/IrO₂ at the anode electrode achieved the highest lipid and protein extraction of 3.27% g_{Lip}/g_{dry wt} and 39.91 mg/L respectively. In addition, the lowest energy consumption also observed from Ti/IrO₂ as lower as 8.58 kWh/m³. Table 2 shows the summary of the electrochemical treatment for *C. vulgaris* lipid extraction. Using Ti/IrO₂ as the anode electrode was better compared to other materials. This because, Ti/SnO₂ has high crystalline nature and Ti/PO₂ has weak interaction with the hydroxyl group [33].

Table 2. Summary of electrochemical treatment in microalgae.

Microalgae strain	Purpose of Study	Electrode		Lipid (% g _{Lip} /g _{dry wt})/ Proteins (mg/L)	Electric Field Strength, E (V/cm)	Reference
		Cathode	Anode			
<i>Chlorella vulgaris</i>	Extraction of lipid and protein	SS	Ti	2.65/19.43	30.7	[33]
<i>Chlorella vulgaris</i>	Extraction of lipid and protein	SS	Ti/IrO ₂	3.27/39.91	14.3	[33]
<i>Chlorella vulgaris</i>	Extraction of lipid and protein	SS	Ti/SnO ₂	2.76/15.11	30.5	[33]
<i>Chlorella vulgaris</i>	Extraction of lipid and protein	SS	Ti/PbO ₂	2.08/17.12	26.3	[33]

Note: SS represents stainless steel electrode, Ti represents Titanium electrode, IrO₂ represents Iridium (IV) Oxide electrode, SnO₂ represents Tin (IV) Oxide electrode, PbO₂ represent Lead (IV) Oxide electrode.

5. Conclusion

In conclusion, biodiesel from microalgae as an alternative feedstock for renewable energy is promising, not only it is eco-friendly and promotes clean energy, but also capable in reducing or eliminating carbon dioxide from the atmosphere. Nevertheless, extraction technology is one of the major challenges to obtain a high yield lipid from microalgae. Several conventional methods found to be efficient, but also have limitations. Cell electroporation of microalgae using PEF and electrochemical treatment methods are both demonstrated to have potential and promising results in increasing the extraction efficiency. Besides, both methods consume less energy and no additional chemicals are required for the system.

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