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A Feasibility Study Using Electrolysis Treatment (ET) As the Pre-treatment Method to Extract Lipid from *Chlorella* sp. for Biodiesel Production

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ABSTRACT

The feasibility study on *Chlorella* sp. lipid extraction using an electrolysis treatment (ET) as pre-treatment was investigated. Stainless steel was used as the anode and cathode material. The ET method was conducted in a batch or continuous system with or without air aeration and recycling flow. The total lipid in *Chlorella* sp. A_{WET} and A_{WET} were not analysed due to small sample volume. Approximately same amount of lipids were attained from *Chlorella* sp. B_{WOET} ($7.97 \pm 0.43\%$ $g_{lipid}/g_{dry\ wt}$) and B_{WET} ($7.95 \pm 0.37\%$ $g_{lipid}/g_{dry\ wt}$) if treated at 5 V/cm and aerated at 16.7 $\mu m^3/s$ for 1800s. Whereas, if *Chlorella* sp. was treated at 13 V/cm and aerated at 16.7 $\mu m^3/s$ for 1800 s, the total lipid obtained in *Chlorella* sp. C_{WOET} ($8.18 \pm 0.49\%$ $g_{lipid}/g_{dry\ wt}$) was 1.13-fold higher than C_{WET} ($7.22 \pm 0.47\%$ $g_{lipid}/g_{dry\ wt}$). Meanwhile under semi-continuous system, similar pattern of result was achieved in *Chlorella* sp. D_{WOET} ($8.58 \pm 0.49\%$ $g_{lipid}/g_{dry\ wt}$) with 1.11-fold higher than D_{WET} ($7.72 \pm 0.54\%$ $g_{lipid}/g_{dry\ wt}$), if treated at 14 V/cm and recycled at 2.3 $\mu m^3/s$ for 3000s. This corresponded to lipid oxidation that might have occurred during the ET method. The fatty acid methyl ester (FAME) composition of *Chlorella* sp. D_{WOET} and D_{WET} contained predominantly methyl linolenate (C18:3) and methyl palmitate

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(C16:0). The concentrations of methyl palmitate attained in *Chlorella* sp. D_{WOET} and D_{WET} were 0.049 ± 0.005 g/m^3 and 0.045 ± 0.005 g/m^3, respectively.

Keywords: *Chlorella* sp., electrolysis treatment, lipid extraction, methyl palmitate, total lipid

INTRODUCTION

This depletion of fossil fuel, in addition to fluctuations in oil prices and global warming issues, makes the development of alternative and renewable resources of energy crucial – a need reflected in a 6.5% increase in biodiesel production worldwide in 2016 (BP Statistical Review of World Energy, 2017). Biodiesel from microalga has received considerable attention over the last decade. Microalgae have many desirable features: a flexibility to grow in various conditions, a high growth rate, lipid-rich content, non-seasonal production type, non-arable land requirement, and non-food crop status. These attractive properties have driven interest in the use of microalgae as a substitute for conventional biodiesel feedstock (Japar et al., 2017; Kumar & Sharma, 2016; Patel et al., 2017; Suali & Sarbatly, 2012).

The process of producing biodiesel from microalgae involves microalgae cultivation, microalgae harvesting, microalgae lipid extraction and transesterification of lipid to fatty acid methyl esters (FAME) (Japar et al., 2017; Mansa et al., 2012; Velasquez-Orta et al., 2013). Of all the processes, lipid extraction is one of the most complex and challenging. Conventional extraction technologies such as high pressure homogenizing, supercritical carbon dioxide extraction, sonication, microwave extraction, osmotic shock, solvent extraction and enzymatic degradation are commonly used in microalgae lipid extraction (Chen et al., 2016; Jeevan-Kumar et al., 2017). Some researchers have also conducted a pre-treatment based on mechanical or physical extraction method as a purpose to disrupt the microalgae rigid cell wall and followed by the solvent extraction method for easy access of the microalga lipid (Joannes et al., 2015a). These methods are found to be efficient but may have several drawbacks such as high energy and time consumption, high toxicity or flammability, expensive raw materials, and difficulty in scaling up (Günerken et al., 2015).

A recent development in extraction technology using electric fields to disrupt the cell wall of microalgae has drawn interest for the last decade such as pulsed electric field (PEF) (Grimi et al., 2014; Lam et al., 2017; Parniakov et al., 2015; Postma et al., 2016). The microalga lipid extraction can be extracted via PEF alone and improved using PEF prior to solvent extraction method (Joannes et al., 2015a). Although, the PEF extraction process is conducted in a very short time period it involves a very high voltage (0.075 - 45 kV) (Joannes et al., 2015b). Therefore, extra precautions are mandatory if using PEF. Aside from PEF, another new electrical based approach to microalgae lipid extraction is the use of a direct current (DC) at a lower voltage such as electrolysis treatment (ET) method (Daghrir et al., 2014; Guldhe et al., 2016; Hua et al., 2016; Misra et al., 2015, 2014). The method is conducted under a batch system in microalgae harvesting stage (Guldhe et al., 2016;

Misra et al., 2015, 2014; Zhou et al., 2016). Moreover, electrolytes such as sodium chloride and sodium sulphate are usually being added into the sample to enhance its conductivity.

As described by Uduman et al. (2010) the electrodes with the same or different materials are submerged into a container which consist of microalga and then the electric is applied. This creates a constant electrical potential difference between the positive (anode) and negative terminal (cathode). The microalga cells (negatively charged) will tend to coagulate at anode (Uduman et al., 2010). This phenomenon also called as electrophoresis or the movement of small, suspended particles in a liquid via potential differences between the electrodes (Barros et al., 2015). In other theory, similar to PEF the electric field induction will causes a phenomenon called reversible or an irreversible electroporation of the cell membrane (Dimitrov, 1995; Kotnik et al., 2012). It is believed that, every cell membrane has its own critical strength. When the electric fields strength is increased above the critical strength of the cell membrane, the forces of noncovalent interaction that binds the lipid bilayer together will be overcome by the electric field forces (Kotnik et al., 2012). This will then lead the formation of small pores or holes on the surface of the cell membrane. Exposing the cell to very high field strength will cause the enlargement of pores and enhance the pores number. Thus, resulting in the released of intracellular components such as lipid, proteins, carbohydrates.

Hence**,** the aim of this work is to investigate the feasibility of using the electrolysis treatment (ET) method and without the addition of any electrolyte to extract microalgae lipid for biodiesel production. This work is used a direct electrolysis (without any additional of electrolytes) specifically for microalgae lipid extraction which has not yet been reported so far. Instead of using non-sacrificial carbon electrodes (Guldhe et al., 2016; Misra et al., 2014, 2015) the present study used stainless steel material for both cathode and anode. Herein, *Chlorella* sp. was pre-treated using ET method prior to solvent extraction. It was expected that, by using ET, the lipid extraction can be improved or enhanced.

MATERIALS AND METHODS

Microalga Strain, Media and Culture Condition

Local freshwater microalga *Chlorella* sp. was obtained from the Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah (Joannes et al., 2016). The cell sizes were in the range 3 – 8 µm. The microalga was cultivated in a 0.002 m^3 Erlenmeyer flask with five batches for 30 days of cultivation under photoautotrophic mode using a Bold's Basal Medium (BBM). The cultivation process was conducted under a florescent white cool lamp with light intensity of 24 $\mu mol/m^2 \cdot s$ and aerated through air bubble. The culture temperature and pH were maintained at 24.4 ± 0.1^oC and 8.89 ± 0.05^oC, respectively. Cultivation was conducted under a cycle of 12 h light and 12 h dark.

Microalga Cell Concentration

The microalga cell concentration was manually determined by dividing the microalga dried weight with its volume and can be referred in Eq. [1]. Approximately 20 μm^3 of microalga culture was taken using a pipette and was placed in a 25 μm^3 container. The initial mass of the container was weighed using an analytical balancer with an accuracy of ± 0.0001 g. Next, the sample was placed in an oven at 70°C and left overnight to dry. Before recording its final mass, the sample was placed in a desiccator and cooled at a room temperature for 2 to 3 mins. Then, the final mass of the container was recorded. The sample was placed into the oven again for an additional 30 mins and reweighed until a constant reading was obtained. The above steps were repeated twice. This was to ensure the final weight of the dried microalga was achieved.

$$\text{Microalga concentration} = \frac{\text{Dried weight (kg)}}{\text{Volume } (m^3)} \times 100\% \quad [1]$$

Experimental Parameters and Chamber Design Setup

The schematic diagram of the electrolysis treatment (ET) chamber was shown in Figure 1. The ET chamber was made of 0.0025 m thick glass (soda-lime-silica glass). Its dimensions were 0.174 m (length) × 0.12 m (height) × 0.051 m (width). Stainless steel with dimensions of 0.154 m (length) × 0.109 m (width) and 0.0004 m thickness was used as the electrode material in anode and cathode. The gap between the electrodes was fixed at 0.022 m. The length and inner diameter of the tube used in this study were 1.5 m and 0.004 m, respectively. The electrodes were connected to a DC power supply (MP 303-3, Meguro, Malaysia). A peristaltic pump was used to create the recycling flow at 2.3 $\mu m^3/s$ and was measured using a digital flowrate meter. The aeration flow was formed using a bubble stone at 16.7 $\mu m^3/s$. Volume of sample used were 60 μm^3 for A sample and 0.5 μm^3 for B, C, D, H_1, H_2 and H_3 samples. The microalga cell concentrations used were in the range 0.18 – 0.46 kg/m^3. Voltages from 11 to 31 V were applied to form an electric field between the

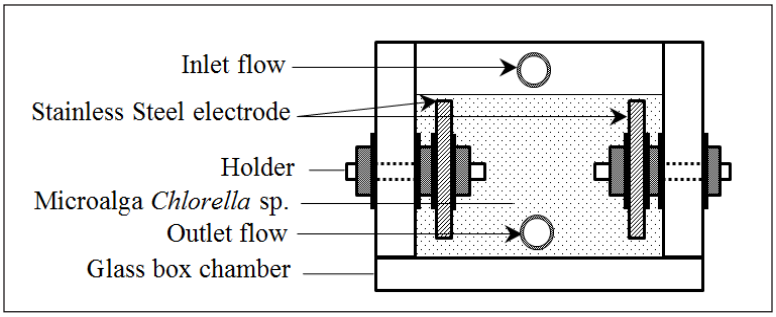


Figure 1. Schematic diagram of the ET chamber (front view)

electrodes of 5 – 14 V/cm. The experiment (A, B and C condition) was conducted under a batch system except for D condition which was conducted under a semi-continuous system. The experiment was conducted in duplicate and all values presented were in average ± standard errors unless stated.

Direct Heat Treatment Setup

A 0.0005 m^3 of *Chlorella* sp. labelled as H_1, H_2 and H_3 were placed in a beaker, stirred and heated at 30°C, 45°C and 60°C using a magnetic stirrer (HS0707V2, Favorit, Malaysia) for 1800s, respectively. A digital thermometer was also used to double check the temperature of the sample.

Lipid Extraction and Total Lipid Determination

After the ET method, the sample was left overnight to allow the formation of three layers: microalgae biomass at the bottom layer, water in the intermediate layer, and unknown components in the upper layer. *Chlorella* sp. without electrolysis treatment (WOET) and with electrolysis treatment (WET) samples were centrifuged at 8000 rpm for 15 mins, frozen at -78°C overnight, and then freeze-dried at -41 ˚C under vacuum conditions for 24 h. The total lipid was extracted from the lyophilized biomass via the Folch method (using a solvent mixture of 13.3 μm^3 of chloroform and 6.7 μm^3 of methanol, $CHCl_3$-MeOH; 2:1; v/v) (Folch et al., 1957) and left for 12 h without stirring. The biomass was filtered using filter paper and the filtrate was washed using 4 μm^3 of water to separate the $CHCl_3$ (bottom layer) and MeOH-water (upper layer). The bottom layer was extracted using a Pasteur pipette, evaporated at 62°C using an evaporator and the residue was dried at 70°C for 2 h. The sample was left to cool at room temperature and the final weight of the extract was recorded. The total lipid content was calculated using Eq. [2] (Abdullah et al., 2017).

$$\text{Total lipid (\%)} = \frac{\text{Weight of lipid (g)}}{\text{Weight of dried microalga (g)}} \times 100\% \qquad [2]$$

Scanning Electron Microscopy

Approximately 10 μm^3 of *Chlorella* sp. from D_{WOET} and D_{WET} samples were harvested and centrifuged at 8000 rpm for 5 mins. Then, the samples were frozen at -78°C overnight. The concentrated cells were lyophilized using a freeze dryer at -4°C under vacuum conditions for 24 h. Next, the surface morphologies of the samples were examined by a scanning electron microscopy (SEM) (EVO MA 10, Carl Zeiss, Germany) (Joannes et al., 2016). The sample was viewed under 1000× and 3000 × magnification powers.

Microalga Lipid Functional Group Determination

Approximately, 500 μm^3 of $CHCl_3$ (containing lipids) from bottom phase was collected using a 1 μm^3 micropipette and placed into a glass vial with cap. A Fourier transform infrared spectroscopy (FTIR) (2000 Series, Perkin-Elmer, UK) was used to determine the functional groups of lipid from D_{WOET} and D_{WET} samples. The wave number from 800 to 2600 cm^{-1} was set to scan the lipid functional groups.

FAME Conversion Procedures

The extracted lipid was converted to fatty acid methyl esters (FAME) using the method and procedures described by Zbinden et al. (2013). First, 0.5 μm^3 of a mixture of MeOH and toluene (1:1; v/v) was added to dissolve the dried lipid sample. Toluene was used to solubilize the non-polar lipids (e.g. cholesterol esters or triacylglycerol). Second, 0.5 μm^3 of 0.2 kg/m^3 methanol KOH solution was added. The sample was sealed in a 28 μm^3 universal bottle and vortexed. Next, it was heated at 37°C for 15 min in a water bath to allow the transesterification reaction to occur and then was cooled at room temperature for a few minutes. 0.5 μm^3 of acetic acid solution 0.2 kg/m^3 was added into the sample, followed by 2.0 μm^3 of $CHCl_3$ and 2.0 μm^3 of deionized water (DI). Mixing by swirling was performed for each addition of the reagents. The sample was then centrifuged at 1500 rpm for 5 mins. After the two layers were formed, the bottom layer ($CHCl_3$ phase) was extracted using a Pasteur pipette and was placed into a new and clean universal bottle. Finally, 1.0 μm^3 of $CHCl_3$ was added into the sample again for further purification and the sample was centrifuged once more, extracted, and placed into a new universal bottle. These steps were repeated twice. The extracted sample was to be further tested in a GCMS for FAME content.

GCMS Analysis Procedures

The analysis of FAME was performed using Gas chromatography mass spectrophotometer (GCMS) (Model 6890N, Agilent Technologies, USA). First, 1 μm^3 of the sample was injected into the GC capillary of 30.0 m × 0.25 mm × 0.25 µm ID nominal. The GCMS setting was adapted from Zbinden et al. (2013) but used zero holding time at maximum temperature. The flow rate of Helium gas (the carrier gas) was set at 1.4 μm^3/min with an initial temperature of 120°C held for 2 mins, then increased 6°C/min to 180°C, 1.5°C/min to 198°C, 5°C/min to 240°C. The split ratio was set at 120:1. The methyl palmitate (n-hexadecanoic acid methyl ester) standard was used as an external standard for FAME and was purchased from Sigma-Aldrich (≥ 99% purities). The methyl palmitate standard was prepared with five different concentrations ranging from 0.36 ± 0.04 $\mu g/m^3$ to 3.63 ± 0.06 $\mu g/m^3$. The concentrations of methyl palmitate in *Chlorella* sp. D_{WOET} and D_{WET}

samples were only determined and quantified using the standard curve of y = 18406792x ($R^2 = 0.995$); where y represents the peak area in cm^2 and x represents the methyl palmitate concentration in g/m^3.

RESULTS AND DISCUSSION

Total Lipid Content Extracted Based On Test Conditions

The total lipid content in each samples was quantified except from A_{WOET} and A_{WET} samples. This was due to sample volume used was too small (60 μm^3), hence not enough for further solvent extraction. From Table 1, it can be seen that the total lipid of 0.39 ± 0.02 kg/m^3 *Chlorella* sp. obtained from the B_{WOET} and B_{WET} were 7.97 ± 0.43% $g_{lipid}/g_{dry\ wt}$ and 7.95 ± 0.37% $g_{lipid}/g_{dry\ wt}$, respectively. These values were approximately the same. Meaning to say, using the ET method at 5 V/cm for 1800s and with an air aerated at 16.7 $\mu m^3/s$ was not able to improve the lipid extraction. The field strength applied might not be enough to weaken the *Chlorella* sp. cells wall. By maintaining the treatment time and aeration flow but then increased the *Chlorella* sp. cell concentration and electrolysis field strength to 0.43 ± 0.01 kg/m^3 and 13 V/cm, respectively. The total lipid extracted from C_{WOET} (8.18 ± 0.49% $g_{lipid}/g_{dry\ wt}$) was found to be 1.13-fold higher than C_{WET} (7.22 ± 0.47% $g_{lipid}/g_{dry\ wt}$). It was also observed that the final temperature of C_{WET} increased from 24.3 ± 0.1°C to 57.2 ± 0.1°C attributed by the increasing of current intensity. Meanwhile, when 0.46 ± 0.01 kg/m^3 *Chlorella* sp. was treated with ET method at 14 V/cm for 3000 s under a semi-continuous system and recycled at 2.3 $\mu m^3/s$. A similar trend of lipid was also attained which showed the total lipid of D_{WET} (7.72 ± 0.54 % $g_{lipid}/g_{dry\ wt}$) was 1.11-fold lower compared to D_{WOET} (8.58 ± 0.49% $g_{lipid}/g_{dry\ wt}$). The final temperature of D_{WET} was also increased from 24.4 ± 0.1°C to 59.1 ± 0.6°C which was also corresponded to the increasing of its current intensity during ET experiment.

The cause of low total lipid in C_{WET} and D_{WET} samples than the control sample might be because the treatment conditions used here had provided an extreme condition for the microalga cells. Further investigation regarding the experimental design are needed to clarify the results obtained here. For that reason, this extreme condition might contribute to the lipid oxidation phenomenon which may occur during the ET method (Daghrir et al., 2014). The formation of oxygen gases either from the surrounding or released from the anode terminal, may assist the lipid oxidation mechanism. According to Meullemiestre et al. (2016) the lipid degradation could also be affected by hydrolysis or oxidation mechanism such as photo-oxidation, auto-oxidation or enzymatic-oxidation reaction. It was also presumed that the increasing of the sample temperature might had assisted the lipid oxidation process. In other theory, during ET method the microalga cells wall was destroyed and led the intracellular lipid released to the media, then was removed during centrifugation, hence, lowering the total lipid extracted from these samples. As a matter

Table 1
ET operational parameters and Chlorella sp. total lipid content based on test conditions

Test conditions	Microalga conc. (kg/m^3)	Treatment time (s)	Field strength (V/cm)	Aeration flow ($\mu m^3/s$)	Recycling flowrate ($\mu m^3/s$)	Temperature (°C)		Total lipid (% $g_{lipid}/g_{dry\ wt}$)	Methyl palmitate (g/m^3)
						Initial	Final		
A_{WOET}	0.18 ± 0.01	-	-	-	-	22.5 ± 0.1	-	NA	NA
A_{WET}	0.18 ± 0.01	600	13	-	-	22.5 ± 0.1	29.0 ± 0.6	NA	NA
B_{WOET}	0.39 ± 0.02	-	-	-	-	24.3 ± 0.1	-	7.97 ± 0.43	NA
B_{WET}	0.39 ± 0.02	1800	5	16.7	-	24.3 ± 0.1	38.0 ± 0.9	7.95 ± 0.37	NA
C_{WOET}	0.43 ± 0.02	-	-	-	-	24.3 ± 0.1	-	8.18 ± 0.49	NA
C_{WET}	0.43 ± 0.02	1800	13	16.7	-	24.3 ± 0.1	57.2 ± 0.1	7.22 ± 0.47	NA
D_{WOET}	0.46 ± 0.01	-	-	-	-	24.4 ± 0.1	-	8.58 ± 0.49	0.049 ± 0.005
D_{WET}	0.46 ± 0.01	3000	14	-	2.3	24.4 ± 0.1	59.1 ± 0.6	7.72 ± 0.54	0.045 ± 0.005

Note: A_{WET} : Batch system without an air aeration flow and recycling flow; B_{WET} : Batch system with an air aeration flow but without recycling flow; C_{WET} : Batch system with an air aeration flow but without recycling flow; D_{WET} : Semi-continuous system without an air aeration flow but with recycling flow; NA: Not analysed

of fact, the trend of result obtained in this study was also similar to the results gained by Daghrir et al. (2014). They had also found that the total lipid of *C. vulgaris* WOET sample was 6.23 ± 1.05% $g_{lipid}/g_{dry\ wt}$ which was higher than WET sample about 5.53 ± 0.23% $g_{lipid}/g_{dry\ wt}$. They used an electrochemical treatment method which an adaptation of electrolysis method to extract lipid from *C. vulgaris*. Instead of using stainless steel, titanium (Ti) insulated with iridium (IV) oxide (Ti/IrO_2) was used as the anode material. The material promotes good interaction between the hydroxyl and the Ti/IrO_2 surface has a good mechanical strength compared to stainless steel (Zaviska et al., 2011). However, the market price of stainless steel is 1.4 times cheaper than Ti (Great American Group, 2015). Hence, this is one of the reasons stainless steel was used as the electrode material and may contribute to lower production costs when using the ET method.

Comparison of Total Lipid Extracted Using Electrical Based Approach

Table 2 shows the comparison of total lipid extracted from five microalgae species including the extractions data based on pervious reported literatures and the present study. Based on these data, the highest total lipid extracted that attained was 26.37 ± 0.47% $g_{lipid}/g_{dry\ wt}$ from *A. falcatus* which was 3.4 times higher than the total lipid obtained in this study (Misra et al., 2014). This might due to the higher microalga concentration (2.88 kg/m^3) that was used. Moreover, the cell size of *A. falcatus* was much bigger than *Chlorella* sp. cell. In fact, the electrode type (i.e. non-sacrificial carbons) used was also different and noted that the conductivity of carbon (5.9×10^6 S/m) was 4 times higher compared to stainless steel (1.37×10^6 S/m) (TIBTECH). By all means, higher conductivity would provides a good efficiency of the cell membrane electroporation (Joannes et al., 2015b).

Hua et al. (2016) used an electrochemical advanced oxidation processes to extract lipid from *S. dimorphus*. The microalga was treated at 8 V/cm and 0.5 A for 7200 s and using stainless steel (cathode) and Ti_4O_7 (anode). The results indicated that the total lipid extracted was increased from 15.2 ± 0.6% to 23.4 ± 0.7% $g_{lipid}/g_{dry\ wt}$. Using the non-sacrificial carbons as the electrodes and applying 1.0 V/cm and 1 A for 1800 s to *A. falcatus*, the highest total lipid can be obtained was 26.37 ± 0.47% $g_{lipid}/g_{dry\ wt}$ (Guldhe et al., 2016). Meanwhile, exposing *S. obliquus* at 1.7 V/cm and 1.5 A for 3600 mins, the highest total lipid achieved was 15.42 ± 0.19% $g_{lipid}/g_{dry\ wt}$. However, decreasing the field strength at 0.77 V/cm and adding 6 kg/m^3 of NaCl (other parameters remained the same), the total lipid of *S. obliquus* extracted was increased to 16.2 ± 0.27% $g_{lipid}/g_{dry\ wt}$ (Misra et al., 2015, 2014). Misra et al. (2014) also found that *C. sorokiniana* lipid could be extracted up to 13.72 ± 0.43% $g_{lipid}/g_{dry\ wt}$ at 0.6 V/cm and 1.0 A for 3600s. Daghrir et al. (2014) had also added 1.42 kg/m^3 of Na_2SO_4 and treated *C. vulgaris* at 14.3 V/cm, 0.6 A and 6000s. In addition, the microalga was recycled at 6.6 $\mu m^3/s$. By using stainless steel at cathode and Ti/IrO_2 at anode, the total lipid extracted was around 5.53 ± 0.23% $g_{lipid}/g_{dry\ wt}$.

Table 2
Comparison of proposed ET method performance with other literatures

Microalga	Parameters	Optimum values	Reference
Chlorella sp.	Initial total lipid	8.58 ± 0.49% $g_{lipid}/g_{dry\ wt}$	Present study
	Final total lipid	7.72 ± 0.54% $g_{lipid}/g_{dry\ wt}$	
	Field strength	14 V/cm	
	Current intensity	1.92 ± 0.02 A	
	Treatment time	3000 s	
	Recycling flow	2.3 $\mu m^3/s$	
	Microalga conc.	0.46 ± 0.01 kg/m^3	
	Electrode type	Stainless steel (cathode and anode)	
	Solvent extraction	Folch method	
Scenedesmus dimorphus	Initial total lipid	15.2 ± 0.6% $g_{lipid}/g_{dry\ wt}$	Hua et al. (2016)
	Final total lipid	23.4 ± 0.7% $g_{lipid}/g_{dry\ wt}$	
	Field strength	8 V/cm	
	Current intensity	0.5 A	
	Treatment time	7200 s	
	Microalga conc.	1.4 kg/m^3	
	Electrode type	Stainless steel (cathode), $Ti4O_7$ (anode)	
	Solvent extraction	Dichloromethane and methanol	
Ankistrodesmus falcatus	Initial total lipid	Not stated	Guldhe et al. (2016)
	Final total lipid	26.37 ± 0.47% $g_{lipid}/g_{dry\ wt}$	
	Field strength	1.0 V/cm	
	Current intensity	1.0 A	
	Treatment time	1800 s	
	Microalga conc.	2.88 kg/m^3	
	Electrode type	non-sacrificial carbons (cathode and anode)	
	Solvent extraction	Folch method	
Scenedesmus obliquus	Initial total lipid	13.60 ± 0.16% $g_{lipid}/g_{dry\ wt}$	Misra et al. (2015)
	Final total lipid	16.20 ± 0.27% $g_{lipid}/g_{dry\ wt}$	
	Field strength	0.77 V/cm	
	Current intensity	1.5 A	
	Treatment time	3600 s	
	Microalga conc.	2.88 kg/m^3	
	Electrode type	Non-sacrificial carbons (cathode and anode)	
	Solvent extraction	Folch method	
	Electrolyte	6 kg/m^3 of NaCl	
Chlorella sorokiniana	Initial total lipid	12.37 ± 0.17% $g_{lipid}/g_{dry\ wt}$	Misra et al. (2014)
	Final total lipid	13.72 ± 0.43% $g_{lipid}/g_{dry\ wt}$	
	Field strength	0.6 V/cm	
	Current intensity	1.0 A	
	Treatment time	3600 s	
	Microalga conc.	2.8 kg/m^3	
	Electrode type	Non-sacrificial carbons (cathode and anode)	
	Solvent extraction	Folch method	
	Electrolyte	6 kg/m^3 of NaCl	

Table 2 *(continue)*

Microalga	Parameters	Optimum values	Reference
Scenedesmus obliquus	Initial total lipid	15.56 ± 0.06% $g_{lipid}/g_{dry\ wt}$	Misra et al. (2014)
	Final total lipid	15.42 ± 0.19% $g_{lipid}/g_{dry\ wt}$	
	Field strength	1.7 V/cm	
	Current intensity	1.5 A	
	Treatment time	3600 s	
	Microalga conc.	2.8 kg/m^3	
	Electrode type	Non-sacrificial carbons (cathode and anode)	
	Solvent extraction	Folch method	
Chlorella vulgaris	Initial total lipid	6.23 ± 1.05% $g_{lipid}/g_{dry\ wt}$	Daghrir et al. (2014)
	Final total lipid	5.53 ± 0.23% $g_{lipid}/g_{dry\ wt}$	
	Field strength	14.3 V/cm	
	Current intensity	0.6 A	
	Treatment time	3000 s	
	Recycling flow	6.6 µm^3/s	
	Microalga conc.	Not stated	
	Electrode type	Stainless steel (cathode), Ti/IrO_2 (anode)	
	Solvent extraction	Folch method	
	Electrolyte	1.42 kg/m^3 of Na_2SO_4	

From this Table of comparison, different treatments with different microalga species will provide different results. However, from Table 2 it was also observed that the total lipid of untreated microalga was always higher or slightly higher than the total lipid of treated microalga. Hence, further investigation should be implemented (e.g. experimental design parameters and optimization) in order to make the ET method to become feasible to be used in future microalga lipid extraction for biodiesel production.

Effect of Direct Heat Treatment

In order to determine the effect of heat towards the microalga lipid extraction, a simple experiment was conducted. Table 3 shows the amount of total lipid extracted from *Chlorella* sp. with different temperature of treatment. The total lipid extracted at 30°C, 45°C and 60°C were 8.59 ± 0.46% $g_{lipid}/g_{dry\ wt}$, 8.57 ± 0.58% $g_{lipid}/g_{dry\ wt}$ and 8.55 ± 0.57% $g_{lipid}/g_{dry\ wt}$, respectively. It can be concluded that, the total lipid extracted in this experiments were no significant difference. According to Zbinden et al. (2013) microalga cell could not be lysed using a direct treatment at 50°C. However, exposing the microalga cell to higher temperature as high as 70°C could cause the cell to lyse (Madigan et al., 2009). In this study, increasing the temperature up to 60°C would not lyse the *Chlorella* sp. cell wall as the total lipid extracted was approximately the same as the control sample (D_{WOET}). It can be concluded that, direct heating will not cause the disruption of microalga cell but may weaken its cell wall.

Table 3
Effect of direct heat treatment on Chlorella sp. lipid extraction

Test condition	Microalga conc. (kg/m^3)	Temperature (^{o}C)	Total lipid (% $g_{lipid}/g_{dry\ wt}$)
D_{WOET}	0.46 ± 0.01	24	8.58 ± 0.49
H_1	0.46 ± 0.01	30	8.59 ± 0.46
H_2	0.46 ± 0.01	45	8.57 ± 0.58
H_3	0.46 ± 0.01	60	8.55 ± 0.57

Microalga Lipid Functional Groups

The FTIR results were shown in Figure 2. From Figure 2(a) it was observed that a similar FTIR spectrum was obtained from D_{WOET} and D_{WET}. Only water functional group was presented in both samples. This had also indicated that lipid was not spontaneously released to the surrounding (Goettel et al., 2013). However, after performing the solvent extraction method, it was found that the results had confirmed the existence of lipid functional groups from both samples as shown in Figure 2(b). The lipid functional groups detected were C = O bond (1740 cm^{-1}), C – O bond (1380 cm^{-1}) and C – O – C bond (1250–1070 cm^{-1}) in *Chlorella* sp. from D_{WOET} and D_{WET} samples (Dean et al., 2010; Forfang et al., 2017). From these results, it can be stated that the microalga lipid cannot be extract directly if using the ET method alone. Thus, it required solvents to access into the lipid granule and then extracting the lipid from the microalga cells.

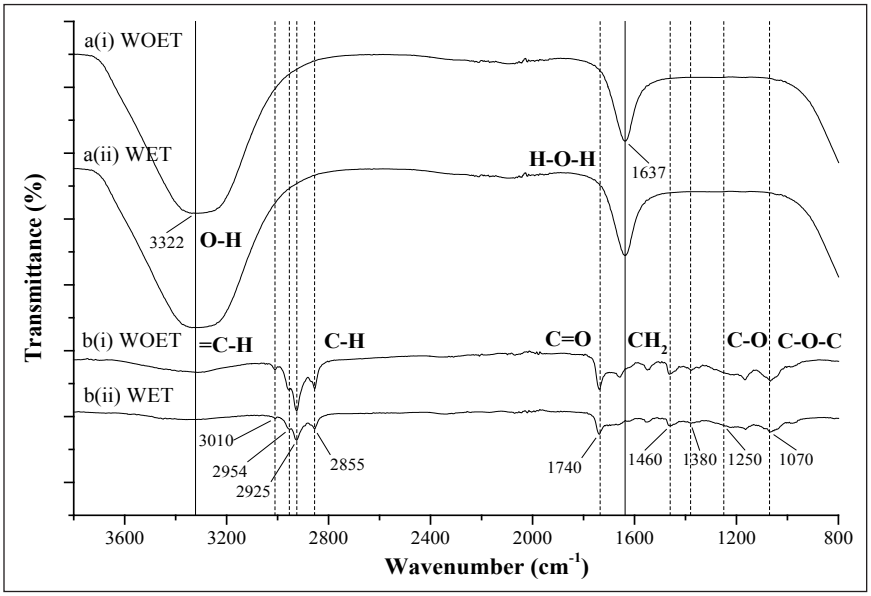


Figure 2. (a) FTIR spectra of (i) *Chlorella* sp. D_{WOET} and (ii) *Chlorella* sp. D_{WET} prior to solvent extraction. (b) FTIR spectra from (i) *Chlorella* sp. D_{WOET} and (ii) *Chlorella* sp. D_{WET} after solvent extraction

Surface Morphology

Figure 3 shows the surface morphology of *Chlorella* sp. cells from D_{WOET} and D_{WET} samples. The cells in Figure 3(a) and 3(b) clearly demonstrated a distinct cell envelope whereas the cells in Figure 3(c) to 3(f) show cell fragments and were agglomerated. The result obtained here was different from the result reported by Yap and co-workers that have observed unshrunk *Chlorella* sp. cells with spherical shape (Yap et al., 2014). The microalga cells in Figure 3(a) and 3(b) appeared to be shrunk and shrivelled due to the freeze drying effect

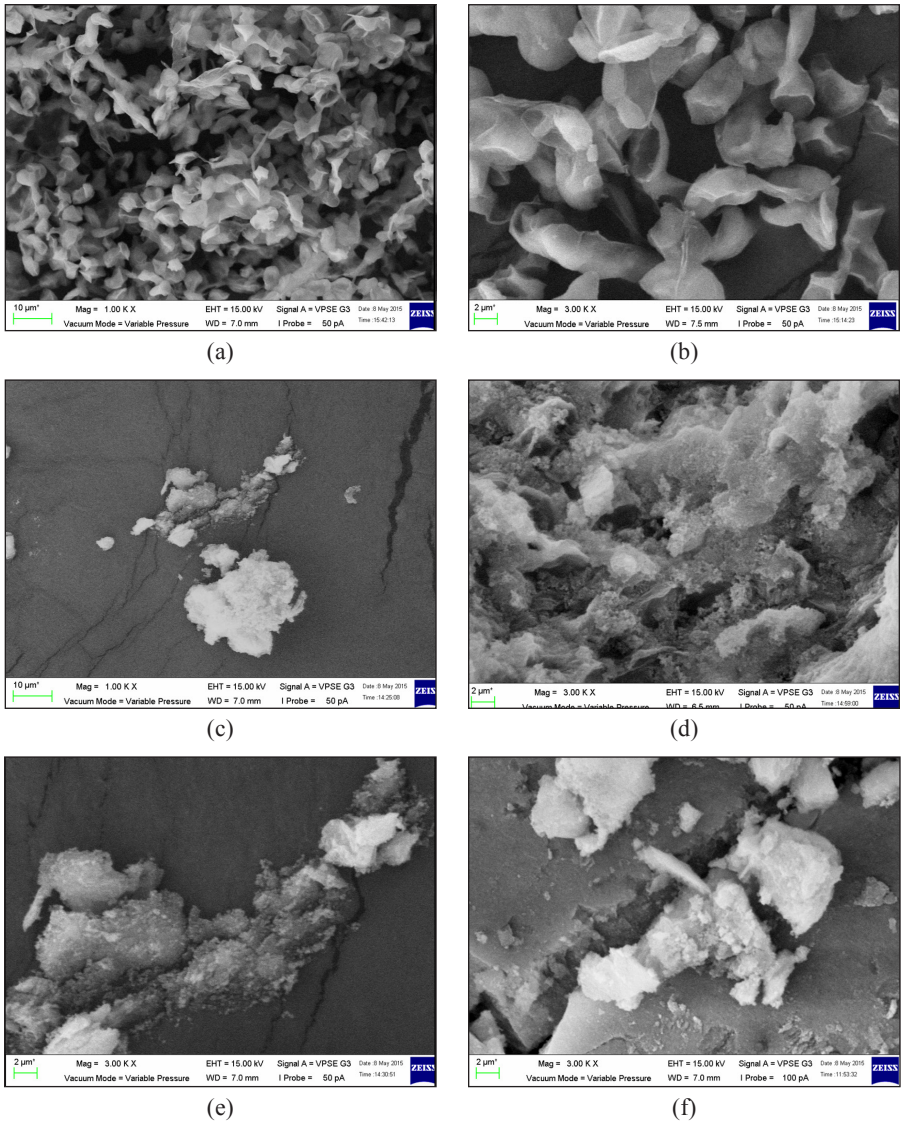


Figure 3. Freeze-dried *Chlorella* sp. cells from D_{WOET} sample viewed under a SEM: (a) at 1000×; and (b) at 3000× magnification power. Freeze-dried *Chlorella* sp. cells D_{WET} sample viewed under a SEM: (c) at 1000×; and (d, e, f) at 3000× magnification power.

(Joannes et al., 2016). Although the microalga cells do not have their typical round shape, but the cell membranes appear unbroken and intact. Meanwhile, Figure 3(c) to 3(f) show only cell fragments with no remaining intact cells. It can be concluded that the cells were disrupted by the external potential applied during ET method.

It is important to be able to view the before and after the condition of the cell walls to claim any enlargement of cell membrane pores. Although it was most likely that the treatment completely destroyed the cell membrane over 3000 s, freeze drying could also have had an added effect on the treated cells. Noted that, freeze drying method also contributes the weakening of the cell membrane but the process will not destroy the cells (Lee et al., 2012). Even though, the *Chlorella* sp. cells were fully destroyed by ET method, their lipid was not spontaneously released. Therefore, a solvent was required to extract the lipid remaining inside the cell in a fashion similar to electrochemical treatment and PEF work (Daghrir et al., 2014; Eing et al., 2013).

FAME Compositions

The FAME compounds of *Chlorella* sp. from D_{WOET} and D_{WET} samples were identified using GCMS and predicted based on the National Institute of Standards and Technology (NIST) library database. Table 3 shows the FAME of the *Chlorella* sp. in D_{WET} sample. Based on the GCMS analysis, three significant peaks were obtained at retention times of 15.90, 16.98 and 22.46 as presented in Figure 4. The GCMS profile for D_{WOET} was not reported here due to the similar mass spectrum obtained thus, only the GCMS profile of D_{WET} was presented.

The GCMS profile confirmed the existence of two major components found at retention times of 16.98 and 22.46 as shown in Figure 5(a) and Figure 5(b), respectively. The two

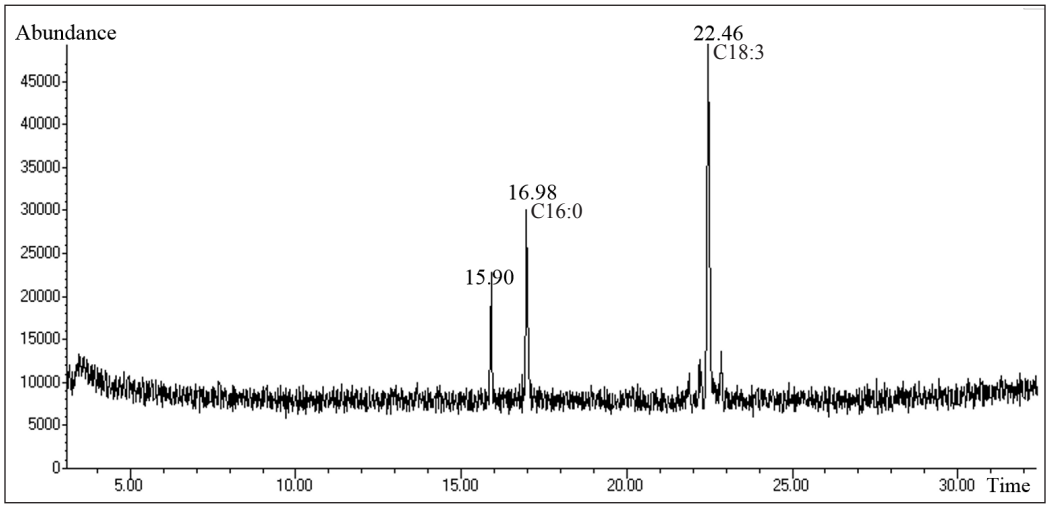
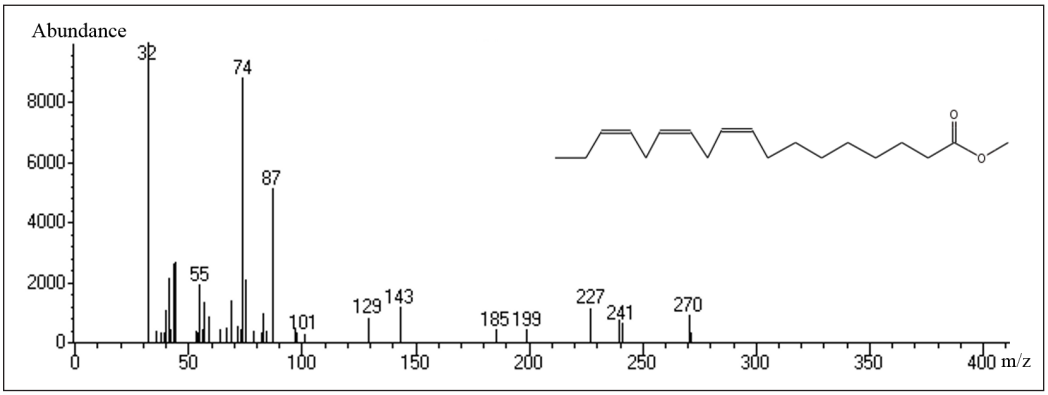
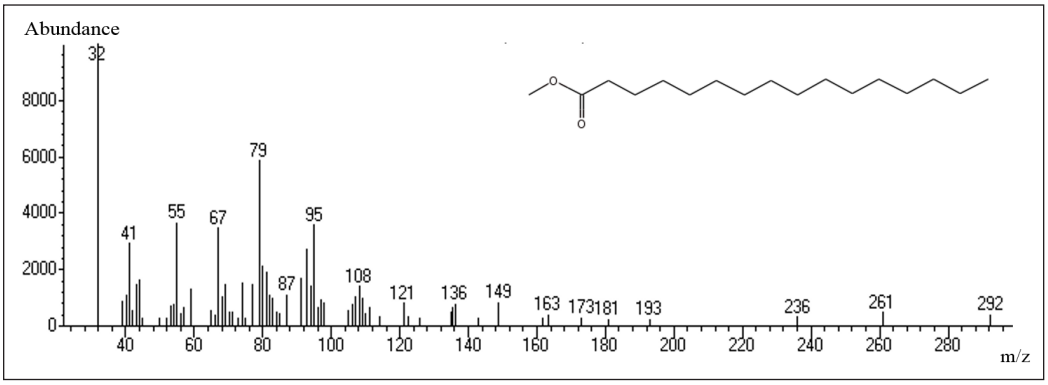


Figure 4. GCMS chromatogram of FAME from *Chlorella* sp. D_{WET}

FAME were methyl palmitate (Hexadecanoic acid methyl ester) and methyl linolenate (Z, Z, Z-9, 12, 15-Octadecatrienoic acid methyl ester). Methyl palmitate is a monosaturated FAME and is mostly present in biodiesel composition, whereas methyl linolenate is a polyunsaturated FAME which has three double bonds unfavourable for biodiesel properties.



(a)



(b)

Figure 5. Mass spectrum of (a) methyl palmitate at retention time 16.98 and (b) methyl linolenate at retention time 22.46 from *Chlorella* sp. D_{WET}

Table 4
FAME components analysed from Chlorella sp. D_{WET} sample

FAME Name	Hexadecanoic acid methyl ester	(Z, Z ,Z)-9,12,15-Octadecatrienoic acid methyl ester
Common Name	Methyl palmitate (C16:0)	Methyl linolenate (C18:3)
Molecular Formula	$C_{17}H_{34}O_2$	$C_{19}H_{32}O_2$
Molecular Weight	270	292
Peak Area (%)	25.97	60.19

From Table 4, it can be seen that methyl linolenate (60.19%) was a dominant component than methyl palmitate (25.97%). Higher levels of polyunsaturated fatty acids will contribute to instability of oxidation in biodiesel engines (Mahapatra et al., 2013). It is important to note that monosaturated fatty acids are preferred for use in biodiesel engines due to their ability to provide a high cetane number for excellent ignition and oxidation stability (Naik et al., 2010; Patel et al., 2017; Rasoul-Amini et al., 2011). It was also observed that this local *Chlorella* sp. lipid was very similar in composition to vegetable oils such as rapeseed (mainly consisting of C16 and C18) and had comparable biodiesel properties (Lang et al., 2001).

The concentration of methyl palmitate extracted from C*hlorella* sp. in D_{WOET} and D_{WET} samples was presented in Table 1. Based on the standard curve of methyl palmitate, the amount of methyl palmitate extracted from the *Chlorella* sp. D_{WOET} and D_{WET} samples were 0.049 ± 0.005 g/m^3 and 0.045 ± 0.005 g/m^3, respectively. In term of number, the amount of methyl palmitate attained *Chlorella* sp. D_{WOET} was slightly higher than in *Chlorella* sp. D_{WET} can be corresponded to the higher total lipid was also attained from the sample (Mansa et al., 2018). However, these values showed that there was no significant difference in methyl palmitate concentration in both samples. The upper layer of the *Chlorella* sp. in D_{WET} sample was also tested for lipid determination using the gravimetric method, transesterified and analysed for FAME. Unfortunately, it was found that the upper layer contained insignificant amounts of lipid and FAME, indicating that *Chlorella* sp. lipid droplets were not spontaneously released into the upper surface and remained inside the cells (Eing et al., 2013).

CONCLUSION

In this work, a local Borneo microalga *Chlorella* sp. was chosen as a potential biodiesel feedstock and ET method as cell disruption method prior to solvent extraction. Using ET method described here, the highest total lipid extracted from 0.46 ± 0.01 kg/m^3 *Chlorella* sp. was $7.72 \pm 0.54\%$ $g_{lipid}/g_{dry\ wt}$ with treatment conditions using the field strength of 14 V/cm and recycled at 2.3 µm^3/s for 3000 s. Heating the microalga to 60^{o}C will not destroy the *Chlorella* sp. cell wall. Indicating that the destroyed and agglomerated cells in D_{WET} sample was attributed by the external potential which was the electric fields in ET. The GCMS analysis also revealed that methyl linolenate (C18:3) was dominant compound than methyl palmitate (C16:0). Methyl palmitate was presented at a level of 0.045 ± 0.005 g/m^3 in $D_{WET.}$ *Chlorella* sp. D_{WET} from the upper layer contained insignificant amounts of lipid and FAME, explaining why the lipid droplets were not spontaneously released from the microalga cells. Hence, a solvent extraction method was required to extract the remaining lipid inside the cell wall. The lipid layer was expected to be formed on the upper

layer of the treated sample. However, based on visual inspection and FTIR analysis, this lipid layer was not present. Based on the results attained in this study, it was found that ET method was unable to enhance the lipid extraction of *Chlorella* sp. Even though the expected result was not achieved, there are still lots of improvements that can be done for the ET to become feasible to be used as the pre-treatment method in microalga lipid extraction. For future works, the ET system with or without air aeration and recycling flow should be emphasized. This is to determine which system gives the best result in term of less temperature increment and with high total lipid extraction. Apart from that, the ET parameters such as treatment time, field strength, recycling flowrate and solvent type need to be varied in order to investigate the optimum point of the microalga lipid extraction. The effect of lipid oxidation needs further investigation to understand the correlation between the extracted lipid and loss of lipid.

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