

# Extraction of Protein from Microalgae Using Low Pulsed Electric Field

Salamah Samsu<sup>1</sup>, Fouziah Md Yassin<sup>1</sup> and Jedol Dayou<sup>1\*</sup>

<sup>1</sup>*Energy, Vibration & Sound Research Group (e-VIBS), Faculty of Science & Natural Resources, Universiti Malaysia Sabah, Jln UMS, 88400 Kota Kinabalu, Sabah.*

Extraction of specific biological components from microalgae is often prevented by the intrinsic rigidity of the cell wall. Therefore, cell disruption is required to permit access to the internal components of the cells. The purpose of this study is to extract protein from microalgae using low Pulsed Electric Field (PEF). *Chlorella vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. were treated with PEF (80 V/cm) in silver/silver and stainless-steel/stainless-steel parallel electrodes treatment chambers for 10 minutes. Treated samples went through solid/liquid separation by centrifugation and the supernatants were further analyzed by Fourier Transform infrared (FTIR) spectroscopy. Results showed that overheating occurred at the negative terminal of the electrode causing damages to cells during treatment. Low pulse duty cycle was able to reduce the overheating effect during PEF treatment. From FTIR spectra, cellulose and protein were detected in the supernatant, indicating that the cells were successfully lysed and some protein managed to diffuse out of the cells during the treatment.

**Keywords:** Cell Disruption, Protein Extraction, Parallel Electrodes, FTIR Spectroscopy

## I. INTRODUCTION

Microalgae have been exploited for decades for its capacity of accumulating protein (Safi et al., 2014). It is known as an efficient biological producer of oil and a versatile source of biomass due to its higher photosynthetic efficiency, biomass productivities and growth rate compared to other mainland crops (Pragya et al., 2013). It grows in an open pond or a closed photo-bioreactor (PBR) (Chisti, 2007; Suali & Sarbatly, 2012). Many studies mainly investigate on lipids extraction for biofuel purposes (Gouvela & Olivera, 2009; Cooney et al., 2009; Foltz, 2012; Azad et al., 2015; Ribeiro

et al., 2015; Guo et al., 2015), thus, neglecting the potential of microalgae as a source of other valuable biological components (Cooney et al., 2009; Kempkes, 2016).

In general, the process of extracting biological components from microalgae includes algae cultivation, harvesting and extraction process from algal biomass (Foltz, 2012). The major setback of the extraction is the algae itself has evolved to protect the contents of the cell. To overcome this barrier, cell disruption is necessary to permit more access to the internal components of the cells. Existing cell wall disruption techniques includes bead milling, sonication, grinding, osmotic shock (Pragya et al., 2013), enzymatic treatment, cell

\*Corresponding author's e-mail: jed@ums.edu.my

homogenizer (Safi et al., 2014) and pulsed electric field (PEF) (Foltz, 2012). Among these, PEF has the greatest potential as it is a non-invasive method that minimizes solution contamination and avoids excessive heating to enable successive cell wall disruption (Kempkes, 2016).

The present study focuses on extracting protein from microalgae by using PEF method, a non-thermal method that involves treatment of samples by applying continuous electrical pulses across a liquid to open the cell membranes of plant cells (Kempkes, 2016).

## II. MATERIALS AND METHODS

In this study, *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Chlorococcum* sp. were used. They were cultivated using Bold Basal Medium (BBM) in an indoor 1L conical PBR at pH value range between 6.4 – 7.0 and temperature between 23 – 28°C (Abdullah et al., 2007; Krohn et al., 2011). Culture mixing was achieved by air pump installed on the top of the PBR to generate current that ensure the microalgae to stay afloat for easier gas exchange at the surface of the culture solution (Persoone et al., 1980). Microalgae were harvested and concentrated by centrifugation at 10,000 rpm for 10 minutes.

The treatment chamber (see Figure 1) consists of two parallel flat electrodes separated 0.5 cm, fixed on glass plate using non-conductive glue. Two types of chambers were used in this study with Silver (Ag) and Stainless

Steel (SST) electrodes pair of parallel Ag/Ag and SST/SST as the disruption process of the microalgae is influenced by the type of electrode material (Daghrir et al., 2014).

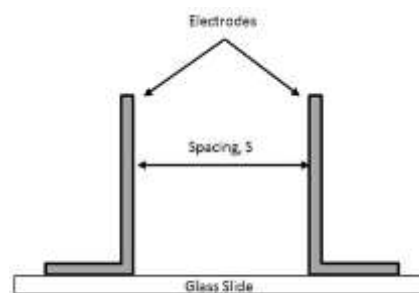


Figure 1. PEF treatment chamber

Concentrated microalgae culture was diluted in distilled water as treatment media. The mixture was then put in the space between the two electrodes. Continuous square electrical pulses of 80 V/cm with duty cycles of 32% and 60% were applied across the parallel electrodes of the treatment chamber for 10 minutes. After that, liquid/solid separation was conducted by centrifugation at 10,000 rpm for 10 minutes at room temperature for 90% efficiency (Pragya et al., 2013). A Perkin Elmer 2000 Series Fourier transform infrared (FTIR) spectrometer was used for scanning all samples (supernatant) for protein detection (Bartosova et al., 2015). The spectrum resolution was set at 4 cm<sup>-1</sup> and the scanning range was selected from 650 to 4000 cm<sup>-1</sup>. Each sample (~100µL) was placed onto the FTIR sample holder and spectra were collected.

## III. RESULTS AND DISCUSSIONS

The PEF waveform produces square pulses with electric field strength of 80 V/cm, frequency of 384.6 Hz and duty cycles of 32% and 60% respectively, as shown in Figure 2.

There were voltage drops caused by the loss of energy and resistance of electrodes during the treatment. The treatment was carried out under lab temperature of 22°C.

Bubbles were formed at the negative terminal of both chambers after 1 minute into the treatment which may indicate microalgae cell damages (Foltz, 2012) when using PEF at 60 % duty cycle. Slight increase in temperature was also detected, reaching to 27°C. The bubbles formation were reduced when lower duty cycle (32%) was used with temperature increasing only to 25°C (see Figure 3). This shows lower pulse duty cycle has reduced the damages to the microalgae cells.

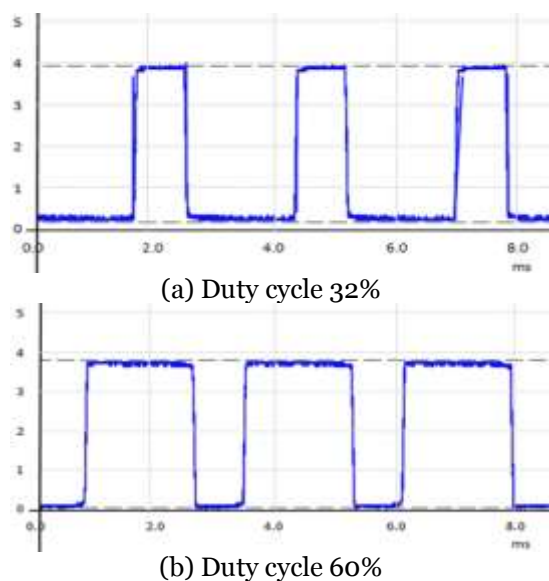


Figure 2. PEF waveforms during treatment

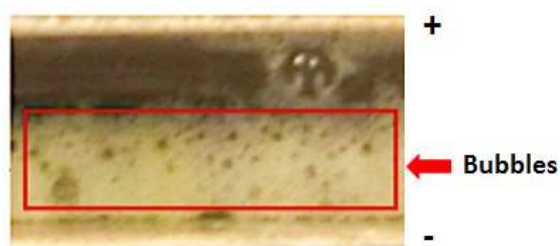
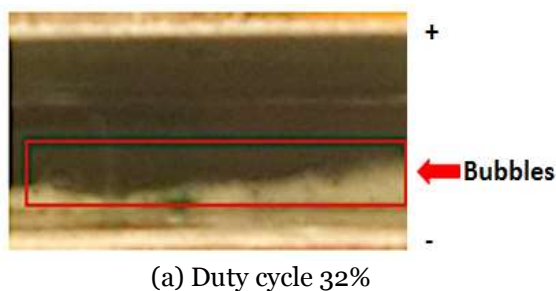


Figure 3. Bubbles formed during PEF treatment

During treatment, more microalgae cells were collected to the positive terminal of the treatment chamber (see Figure 4). Longer exposure of PEF caused inhomogeneity of the algal sample and later formed a non-uniform field. This non-uniform field led to the occurrence of dielectro-poretic force causing more algal cells to move to the positive terminal. Additionally, algal shifting to the positive terminal was also due to the bubble formation on the negative terminal of the electrodes.

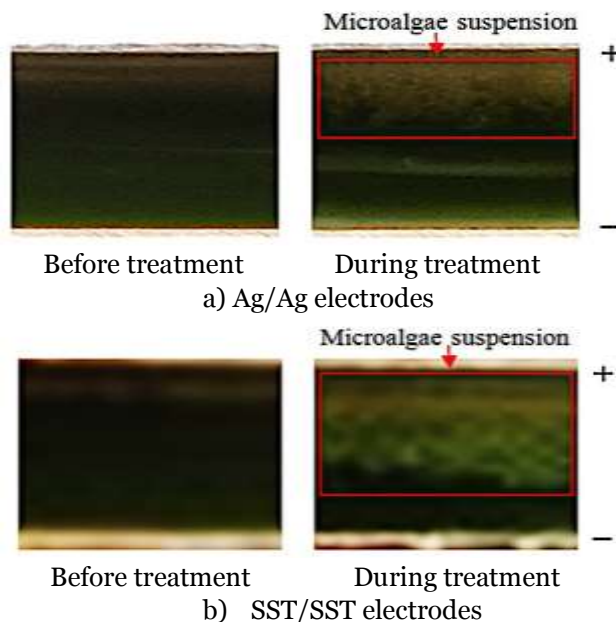


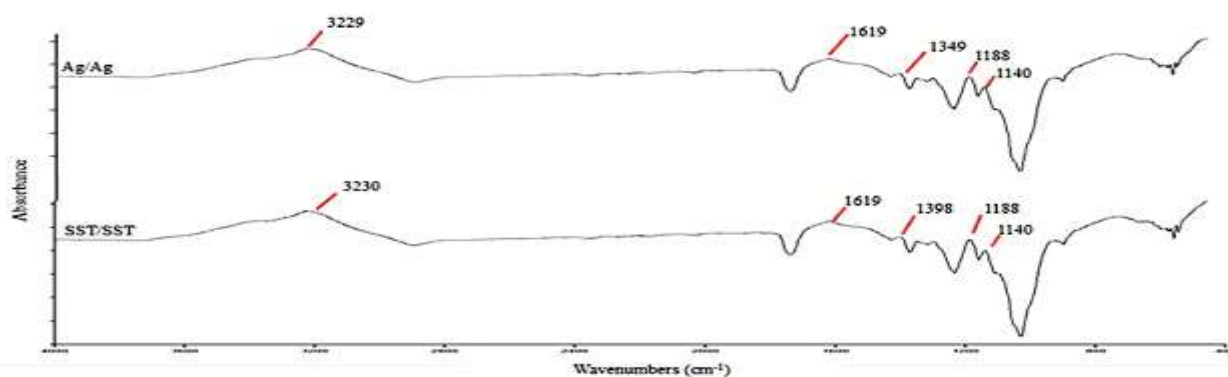
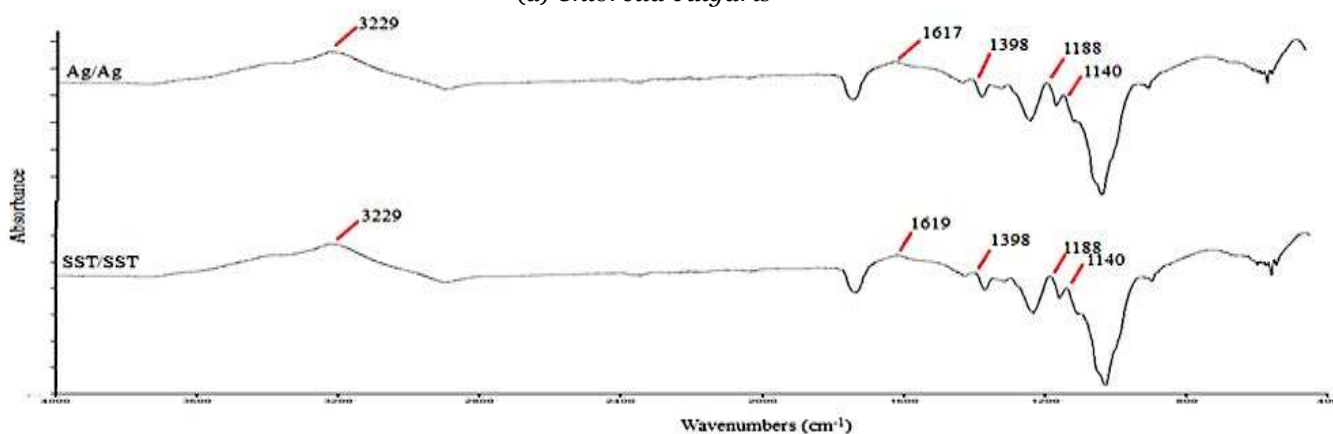
Figure 4. Chlorocella vulgaris during PEF treatment at e-field of 80 V/cm for 10 minutes

FTIR spectra of the extracted material from the microalgae shows that the band was formed by three individual peaks, situated at water and

protein bands ( $3029\text{--}3639\text{ cm}^{-1}$ ), amide I and amide II bands representing proteins (around  $1660\text{ cm}^{-1}$  and around  $1540\text{ cm}^{-1}$ ) and the carbohydrate region ( $1200\text{--}900\text{ cm}^{-1}$ ) (Figure 5). Major peak at  $3229\text{ cm}^{-1}$  was due to O–H stretching (Bartosova *et al.*, 2015) of the water content in the extracted supernatant. The supernatant extracted from the treated sample contained water as it was used as liquid media during the PEF treatment. Carbohydrate absorption also occurred at  $1187\text{ cm}^{-1}$  and  $1140\text{ cm}^{-1}$  peaks mainly due to C–O–C of polysaccharides (Bartosova *et al.*, 2015). One of the main components of the selected microalgae cell wall was cellulose and therefore the presence of polysaccharides may represent this cellulose

cell wall. This was a major indication that the cell wall of the treated microalgae undergone lysis and some part of the cell wall may have been ruptured during the PEF treatment.

The protein spectra could be characterized by the  $1617\text{--}1620\text{ cm}^{-1}$  and  $1349\text{--}1398\text{ cm}^{-1}$  peaks. These peaks were due to mainly C=O stretching vibration and the combination of N–H bending and C–N stretching vibrations in the amide bands, respectively (Bartosova *et al.*, 2015). This indicated that some protein managed to diffuse out from the cells during the cell disruption treatment as the cell wall was lysed and ruptured. This diffusion of protein out of cell was additionally supported by the presence of cellulose in the extracted supernatant.

(a) *Chlorella vulgaris*(b) *Chlorococcum* sp.

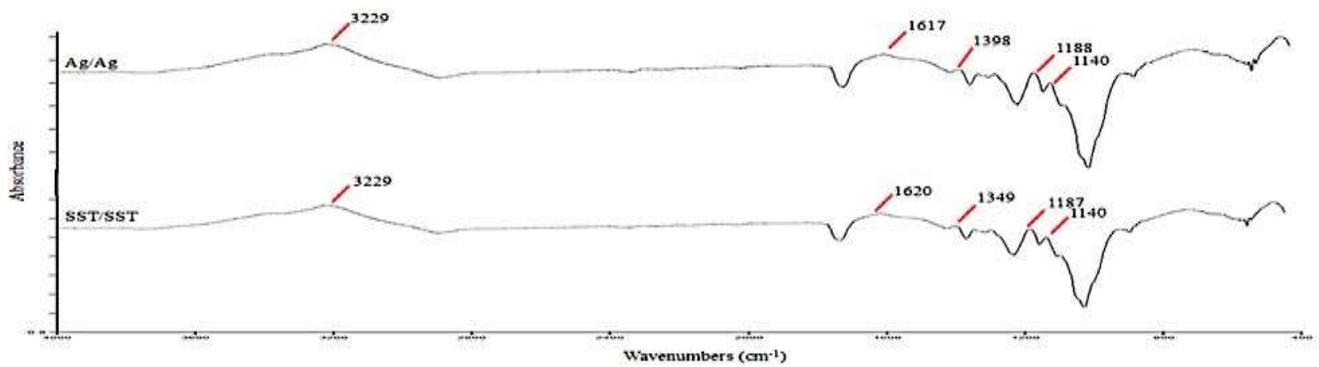
(c) *Scenedesmus* sp.

Figure 5 FTIR spectra of treated microalgae for 10 minutes (80 V/cm; 32% duty cycle)

#### IV. SUMMARY

The present study shows additional insight into understanding the PEF method of cell disruption for protein extraction from *Chlorella vulgaris*, *Chlorococcum* sp. and *Scenedesmus* sp.. FTIR spectra show presence of protein and cellulose in all the extracted supernatant of the samples. At 80 V/cm PEF,

lower duty cycle was shown to be a better choice for cell wall disruption using PEF method as less bubbles was formed which indicates less cell damage.

#### V. ACKNOWLEDGMENT

This work was financially supported by Universiti Malaysia Sabah under UMGreat Grant GUG0011-ST-M-1/2016.

- [1] Abdullah AZ, Razali N, Mootabadi H & Salamatinia B 2007, 'Critical technical areas for future improvement in biodiesel technologies', *Environmental Research Lett*, vol. 2, 034001.
- [2] Azad AK, Rasul MG, Khan MMK, Sharma SC & Hazrat MA 2015, 'Prospect of biofuels as alternative transport fuel in Australia', *Renewable and Sustainable Energy Reviews*, vol. 43, pp. 331-351.
- [3] Bartosova A, Blinova L & Gerulova K 2015, 'Characterisation of polysaccharides and lipids from selected green algae species by FTIR-ATR spectroscopy', *Research Papers of Faculty of Materials Science and Technology In Trnava*, vol. 23.
- [4] Chisti Y 2007, 'Biodiesel from microalgae', *Biotechnology Advances*, vol. 25, pp. 294-306.
- [5] Cooney M, Young G & Nagle N 2009, 'Extraction of bio-oils from microalgae', *Separation & Purification Reviews*, vol. 38, pp. 291-325.
- [6] Daghrir R., Igounet L, Brar SK & Drogui P 2014, 'Novel electrochemical method for the recovery of lipids from microalgae for biodiesel production', *Journal of the Taiwan Institute of Chemical Engineers*, vol. 45, pp. 153-162.
- [7] Foltz G 2012, 'Algae lysis with pulsed electric fields' Master Thesis. California State Polytechnic University, San Luis Obispo.
- [8] Gouvela L & Olivera AC 2009, 'Microalgae as a raw material for biofuels production', *Journal of Industrial Microbiology and Biotechnology*, vol. 36, pp. 269-274.
- [9] Guo M, Song W & Buhain J 2015, 'Bioenergy and Biofuels: History Status and perspective', *Renewable and Sustainable Energy Reviews*, vol. 42, pp. 712-725.
- [10] Kempkes MA 2016, Pulsed electric fields for algal extraction and predator control' In *Handbook of Electroporation*, Springer International Publishing, pp. 1-16.
- [11] Krohn BJ, McNeff CV, Yan B & Nowlan D 2011, 'Production of algae-based biodiesel using the continuous catalytic Mcgyan Process', *Bioresource Technology*, pp. 94-100.
- [12] Persoone G, Morales J, Verlet H & De Pauw N 1980, 'Air-lift pumps and the effect of mixing on algal growth', *Algal Biomass*, pp. 505-522.
- [13] Pragma N, Pandey KK & Sahoo PK 2013, 'A review on harvesting, oil extraction and biofuels production technologies from microalgae', *Renewable and Sustainable Energy Reviews*, vol. 24, pp.159-171.
- [14] Ribeiro LA, da Silve PP, Mata TM & Martins AA 2015, 'Prospects of using microalgae for biofuels production: Results of a Delphi study', *Renewable Energy*, vol. 75, pp. 799-804.
- [15] Safi C, Ursu AV, Laroche C, Zebib B & Merah O 2014, 'Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods', *Algal Research*, vol. 3, pp. 61-65.
- [16] Suali E & Sarbatly R 2012, 'Conversion of microalgae to biofuel', *Renewable and Sustainable Energy Reviews*, vol. 16, pp. 4316-4342.