INVESTIGATION ON PROTEIN EXTRACTION FROM ALGAE USING LOW VOLTAGE PULSED ELECTRIC FIELD

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DECLARATION

I declare that this thesis is the result of my own independent work, except where otherwise stated.

23 JULY 2020

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CERTIFICATION

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ABSTRACT

Extraction of specific biological components like protein from microalgae is often prevented by the intrinsic rigidity of the cell wall. Therefore, cell wall 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 voltage Pulsed Electric Field (PEF). Microalgae *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Chlorococcum* sp. were treated with PEF with electrical field of 380 V/cm in silver (Ag) and stainless steel (SST) parallel plate treatment chambers for 30 minutes. The treated microalgae samples then went through solid-liquid separation by centrifugation. The total protein extracted was quantified using quantitative Bradford Assay. Results showed that overheating occurred at the electrode's negative terminal during the square pulse PEF treatments, causing damages to the cells due to long pulse duration with 60% duty cycle. Lowering the duty cycle or shortening the pulse duration of the PEF pulse reduced the overheating effect during the treatment. This is achieved using exponential decay PEF pulse that enabled longer treatment time up to 30 minutes while minimizing the cell damages. For *Chlorococcum* sp., PEF treatment with electrical field strength 190 V/cm and duty cycle 8% produced higher protein concentration than the other set parameters. Meanwhile, *C. vulgaris* and *S. quadricauda* required longer pulse duration with duty cycle 16% with electrical field strength 190 V/cm or lower duty cycle 8% and electrical filed strength 380 V/cm to allow a higher concentration of extracted protein. All the treated microalgae showed positive viability as they were grown after the treatment. Overall, different cell morphology of the microalgae required particular pulse parameters to ensure successful protein extraction.

ABSTRAK

KAJIAN TERHADAP PENGEKSTRAKAN PROTEIN DARIPADA ALGA MENGGUNAKAN MEDAN DENYUT ELEKTRIK VOLTAN RENDAH

Pengekstrakan komponen-komponen biologi yang spesifik seperti protein daripada mikroalga sering dihalang oleh ketegaran intrinsik dinding sel. Oleh itu, gangguan sel dinding di perlukan untuk membenarkan mengakses ke komponen dalaman sel. Tujuan kajian ini ialah untuk mengekstrak protein daripada mikroalga menggunakan Medan Denyut Elektrik (MDE) voltan rendah. Mickroalga Chlorella vulgaris, Scenedesmus quadricauda and Chlorococcum sp. dirawat dengan MDE dengan kekuatan medan elektrik dengan magnitud 380 V/cm di dalam kebuk rawatan plat-selari Argentum (Ag) dan Keluli Tahan Karat (KTT) selama 30 minit. Sampel mikroalga yang telah dirawat kemudiannya melalui pemisahan pepejal-cecair secara pengemparan. Jumlah protein yang diekstrak diukur menggunakan Ujian Bradford kuantitian. Hasil kajian menunjukkan pemanasan lampau terjadi di terminal negatif elektrod semasa rawatan MDE, menyebabkan kerosakan pada sel yang disebabkan oleh tempoh masa denyut yang panjang dengan kitar tugas 60%. Merendahkan kitar masa atau memendekkan tempoh masa denyut dapat mengurangkan kesan pemanansan lampau semasa rawatan. Perkara ini dicapai dengan menggunakan denyut MDE berbentuk pereputan eksponen yang membolehkan tempoh rawatan dipanjangkan sehingga 30 minit sambil meminimumkan kerosakan sel. Bagi Chlorococcum sp., rawatan MDE dengan kekuatan medan elektrik 190 V/cm dan kitar masa 8% menghasilkan kepekatan protein yang lebih tinggi berbanding set parameter rawatan yang lain. Manakala, C. vulgaris dan S. quadricauda memerlukan tempoh masa denyut yang lebih panjang dengan kitar masa 16% dan kekuatan medan elektrik 190 V/cm atau kitar masa yang lebih rendah 8% dan kekuatan medan elektrik 380 V/cm untuk membolehkan pengekstrakan protein yang lebih tinggi kepekatannya. Kesemua mikroalga yang dirawat menunjukkan kelangsungan hidup yang positif apabila dikultur semula selepas rawatan. Secara keseluruhannya, morfologi sel yang berbeza bagi mikroalga memerlukan parameter denyut yang tertentu untuk memastikan pengekstrakan protein yang berjaya.

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PEF	Pulsed Electric Field
DC	Direct Current
AC	Alternate Current
AG	Silver
SST	Stainless Steel
PBR	Photo-Bioreactor
PM	Plasma Membrane
CW	Cell Wall
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
BSA	Bovine Serum Albumin
BBM	Bold Basal Medium
CGF	Chlorella Growth Factor
GRAS	Generally Regarded As Safe
EFSA	European Food Safety Authority
CHL	Chloroplast
THY	Thylakoids
PFN	Pulse Forming Network
AG/AGCL	Silver/Silver Chloride Electrode
A	Amplitude
T	Period
PWM	Pulse Width Modulation
V	Volt
V/cm	Volt per centimeter
E	Electrical Field Strength
T_0	Initial temperature
T_{30}	Temperature at minute 30

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Microalgae had shown great potential as a protein source (Becker, 2007), an alternative health suppliments for human (Koyande *et al.,* 2019) and renewable energy source (Pragya *et al.*, 2013) such as *Nannochloropsis* sp. and *Neochloris oleabundans* (Gouveia & Oliveira, 2009). In general, the process of valuable biological components from microalgae includes three stages: microalgae cultivation, harvesting and extraction stages (Foltz, 2012).

The most challenging stage of the extraction process was the extraction stage in which the microalgae itself had evolved to protect the inside of their cells. To overcome this challenge, cell disruption was required to permit more access to the internal components of the cells. Some examples of existing disruption methods are solvent extraction method (Gouveia & Oliveira, 2009), sub−critical water extraction method, super−critical fluid method (Cooney *et al.*, 2009), pulsed electric field (PEF) treatment method (Guionet *et al.*, 2015; Parniakov *et al.*, 2015; Guderjan *et al.*, 2007) and many others.

1.1.1 Microalgae Overview

Microalgae is a type of photosynthetic microorganism that has the ability to convert light energy (photon), water and carbon dioxide into algal biomass (Munir *et al.*, 2013). Most microalgae species are reported to contain equal amount of protein compared to traditional protein source (Koyande *et al.,* 2019) and capable of producing energy-rich oil (Kightlinger *et al.*, 2014). In addition, microalgae also offer many advantages over other biomass sources or plants as a source of protein and

biofuel that include high growth rates, high lipid content, have the ability to rapidly improve strains and co-product production (Kightlinger *et al.*, 2014).

Microalgae has a very simple cellular structure and can be found in aqueous environment. For this characteristic, the whole cell surface of microalgae are available for capturing light energy and transfer of mass that leads to high rates of substrate uptake and photosynthetic efficiency (Sheehan *et al.*, 1998; Miao and Wu, 2006).

In addition, microalgae can be cultivated in two types of environments; open system that consists of cultivation in natural waters like lakes, ponds and lagoons or even an artificial ponds and containers that are open to air and close system, also known as photo-bioreactors, that involves cultivation of microalgae in containers, tubes or clear plastic bags (Pulz, 2001). Compare to other land-based crops that needs massive land to be cultivated, microalgae is a better choice.

1.1.2 Valuable Biological Components of Microalgae

Microalgae has gained lots of attention in the past years due to their promising renewable feedstock for protein source (Koyande *et al.*, 2019), health supplements (Becker, 2007), feed, fine chemicals, and biofuel production. Microalgae produce many valuable biological components such as proteins, lipids, pigments, unsaturated fatty acids or polysaccharides considerably at high rates. However, they are mostly stored in the intracellular region and since microalgae have strong cell walls making it harder to extract them. Therefore, cell disintegration is required to penetrate the cell wall and extract all these valuable biological components (Goettel *et al.* 2013; Koyande *et al.,* 2019).

1.1.3 Biological Component Extraction Method

The demand of microalgae proteins and other biological components is increasing over the years and has led to the development of methods for extractions from microalgae. However, there is not a definite method for extraction that has been thoroughly developed. Some examples of those methods are pressing method, solvent extraction method, super-critical fluid extraction method and ultrasonic assisted method (Munir *et al.*, 2013). All these methods involve the rupturing of the cell wall of the samples. Figure 1.1 shows the mechanism of the cell inactivation

process. The process starts from the stage where continuous electric field is subjected to the cells in a medium. After a while, the cells undergo swelling until they reach a maximum swelling size where lysis occurs (Tsong, 1990).

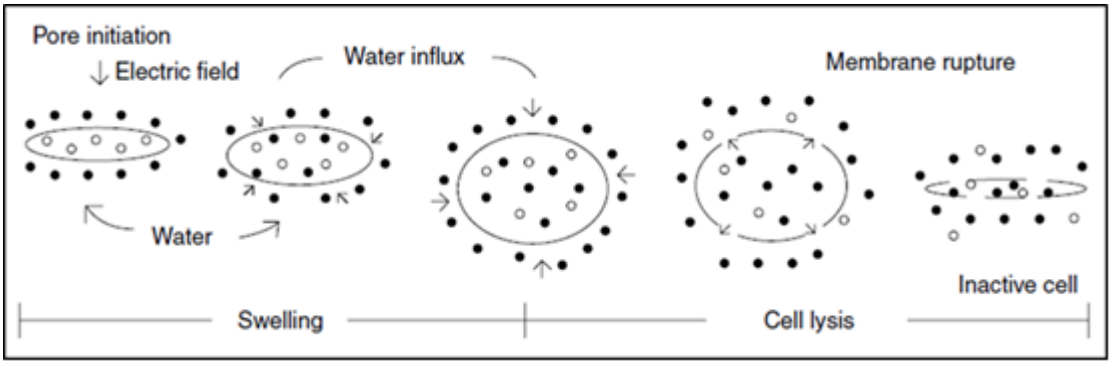

Figure 1-1 Mechanism of cell inactivation
Source: Tsong, 1990

The PEF technology has undergone development in recent years that it is used in food processing and in another field like in biological component extraction such as biomass. Nowadays, this technology is used to extract valuable biological component from microalgae. Four main parameters must be considered to use PEF in the pre-treatment stage of substances extraction from microalgae. They are the electric field strength, electrical pulse duration, type of pulses and the number of pulses. Electric field strength refers to the voltage subjected to the pre-treatment chamber (Zbinden, 2011).

1.2 Problem Statement

Pulsed Electric Field (PEF) had demonstrated a high potential to enhance extraction of valuable components from different biological objects such as proteins and phenolic compounds (Parniakov *et al.*, 2015; Guderjan *et al.*, 2007; Guderjan *et al.*, 2005). It involved the application of short pulses of high voltage in order to disrupt biological cells in the food material (Jaeger, 2012). It was shown that PEF-assisted extraction was highly selective and allowed the release of soluble intracellular matter, while extraction of lipids requires application of solvents (Parniakov *et al.*, 2015). Pretreatment of PEF could be used to enhance cell disruption before oil separation. It was known to affect the quality of the oil extracted from the biological cells, affecting the composition and concentration of minor components of the oil (Guderjan *et al.*, 2007).

High−voltage PEF treatment ranging from 1 kV to tenth of kV had been used to extract biological content from various microalgae such as lipids (Joannes *et al.,* 2015; Eing *et al.*, 2009; Guderjan *et al.*, 2007), pigments, proteins, carbohydrates and phenolic compound (Parniakov *et al.*, 2015). However, the succession of extracting biological content from microalgae depended on the intensity of the PEF treatment that could be defined by the pulse duration, discharge time, number of pulses, pulse energy or electric field intensity. The experiment showed that lysis could be achieved by repetitive lower amplitude or a single pulse of sufficient amplitude (8.0 kV/cm). Therefore, it is possible to optimize energy expenditure by increasing the pulse amplitudedecreasing pulse duration and number; or decreasing the pulse amplitude and increasing the pulse duration or number of pulses (Foltz, 2012).

In this research, the following questions were pursued: What is the optimum pulse amplitude and duty cycle for protein extraction from microalgae? How does it differ for different microalgae species? Therefore, a low voltage PEF treatment is proposed in this research to investigate its effectiveness in protein extraction from *Chlorella vulgaris*, *Chlorococcum* sp. and *Scenedesmus quadricauda*.

1.3 Objectives

The objectives of this research are:

I. To design and test a variable control and low voltage pulse generator ranging from 0 V to 300 V by cascading function generator, voltage amplifier and rectifier blocks.

II. To optimize the electrical field and pulse duty cycle that can be used to extract protein from *Chlorella vulgaris*, *Chlorococcum* sp. and *Scenedesmus quadricauda* at low voltage condition.

III. To develop and use a protein extraction scheme to extract protein from *Chlorella vulgaris*, *Chlorococcum* sp. and *Scenedesmus quadricauda*.

1.4 Scope of Work

All the work plan in this project cover the main development is as listed below:

I. The pulse generator is designed by cascading function generator, boost converter and rectifier circuits to produce a pulse with amplitude of 300 V.

II. Three different green freshwater microalgae species are used for the research purpose: Chlorella vulgaris, Chlorococcum sp, and *Scenedesmus quadricauda.*

III. This research uses two electrodes, silver (Ag) and stainless steel (SST), for the pulsed electric field treatment chamber.

IV. Microalgae samples are treated using pulsed electric field at maximum electric field of 380 V/cm with different pulse characteristics for cell disruption in both treatment chambers for protein extraction.

V. The extracted proteins are analyzed using quantitative Bradford protein assay to determine the total protein content.