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Development of Algal Oil Yield Model for Tetraselmis sp. Extraction Using Pulsed Electric Field (PEF)

Prima Asmara Sejati[1,2*], Yano Surya Pradana[2,3], Ade Kurniawan[2,3], Yosephus Ardean Kurnianto Prayitno[2,4], Thoriq Teja Samudra[2,5], Arief Budiman[2,3,6]

[1]Department of Electrical Engineering and Informatics, Vocational College, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[2]Center for Energy Studies, Universitas Gadjah Mada, Sekip K-1A, Bulaksumur, Yogyakarta, 55281, Indonesia
[3]Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada, Jalan Grafika No. 2, Yogyakarta 55281, Indonesia
[4]Department of Mechanical Engineering, Vocational College, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[5]Faculty of Biology, Universitas Gadjah Mada, Jalan Teknika Selatan, Yogyakarta 55281, Indonesia
[6]Master Program of Systems Engineering, Universitas Gadjah Mada, Jalan Teknika Utara No. 3, Yogyakarta 55281, Indonesia

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ABSTRACT

The extraction of algae oil from *Tetraselmis sp.* using pulsed electric field (PEF) can be evaluated through an extraction yield model developed based on the approximation of lipid mass transfer during the extraction process. Therefore, this study aimed to examine the influence of three primary PEF variables, namely duty cycle (D), frequency (f), and treatment time (t) on the experimental yield. The microalgae samples were extracted within the PEF chamber designed for batch processing, with a maximum volume of 100 mL for each. The chamber consisted of eight stainless-steel plates of a 100x120 mm size and 1 mm in thickness, positioned on a 165x145x42 mm acrylic base. Subsequently, the experimental results were modelled to represent the effect of each variable. The model parameters for maximum yield (grams of extracted lipids per 100 grams of microalgae) were determined as follows: K_0 = 70.7346, yield growth curves factor L=-52.1521, duty cycle percentage factor α = 0.4993, frequency efficiency constant β = 0.7798, and time constant τ = -87.5942. The proposed model exhibited a relatively high coefficient of determination (R_i^2), with R_t^2 =0.9583, R_f^2 =0.9581, and R_D^2 =0.8506, and an average of $\overline{R_i^2}$ =0.9223, respectively.

I INTRODUCTION

The production of biofuels is recently gaining significant attention as a sustainable alternative to fossil fuels [1], [2]. Concerns about fossil energy depletion and the greenhouse effect have spurred efforts to develop biofuels from renewable natural resources [3],

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*Corresponding author.
E-mail address: prima.asmara.s@ugm.ac.id (Prima Asmara Sejati)

[4]. One promising biofuel is biodiesel [5], consisting of esters synthesized from fatty acids found in renewable natural oils and short-chain alcohol, such as methanol or ethanol, in the presence of an acid or base catalyst [6]. Moreover, biodiesel exhibits lower toxicity and greater biodegradability compared to fossil diesel fuel [7]. Based on the potential of biodiesel as a fossil fuel substitute, various biodiesel types sourced from renewable natural oils, including palm, soybean, and sunflower, have been introduced. In Southeast Asia, specifically in Indonesia, palm oil is the most attractive biodiesel feedstock due to its abundance and quality. However, the use of biodiesel feedstocks for energy generation conflicts with the food sector requirements [8]. This leads to the need to explore productive biomass sources that do not compete with food production and require only small land areas [9].

To address the described challenges, microalgae are recommended as a promising biomass source and potential biodiesel raw material. Microalgae are rapid-growing microorganisms with great ability to capture carbon dioxide, producing more oil per acre than other biodiesel feedstocks [10], [11]. These microorganisms thrive on land unsuitable for food crop cultivation, provided adequate light, nutrients, and CO_2 are received for photosynthesis and biomass production [12]. Additionally, their cultivation can be conducted in extreme conditions, such as wastewater, and supplied with CO_2 from flue gas emissions [13]. Each microalgal cell contains varying proportions of carbohydrates, proteins, and lipids, depending on the species [14]. The carbohydrates are often fermented into bioethanol, and the protein content is isolated for food supplement production. Meanwhile, the lipid content can be converted into biodiesel [15]. Due to the potential of microalgae as a biomass source for oil extraction, the application for biodiesel synthesis is challenging.

In the context of biodiesel production from microalgae, four main processes are included, namely cultivation, harvesting, oil extraction, and oil conversion into biodiesel. However, due to the greatest challenge posed by oil extraction, mechanical, electrical, and chemical methods are commonly used for the process [16]. Mechanical extraction incorporates pressure to remove oil from the microalgal cell. In the chemical method, algal oil is extracted with solvents such as *n*-hexane [17], or CO_2, in supercritical conditions [18]. Both mechanical and chemical methods apply dry microalgae as raw materials, leading to energy-intensive and time-consuming drying processes.

To prevent the use of high energy and extensive drying time, electrical energy application has been proposed for oil extraction. Specifically, pulsed electric field (PEF) disrupts microalgal cell walls within an electric field [19]. This method can be effectively combined with chemical extraction to enhance lipid yield from microalgae [20]. The disrupted cells are prone to releasing lipids through electroporation of their membranes, facilitating the extraction of lipids and other cytoplasmic metabolites. This generates a high lipid concentration in the growth media, favouring subsequent chemical extraction processes [21], [22]. Moreover, PEF application to cyanobacteria species such as *Synechocystis* PCC 6803 can completely rupture the cells due to wall absence. This will lead to the release of cellular contents, including lipids, into the growth media, increasing extraction efficiency [23]. Microalgae suitable for PEF generally live in aquatic environments with high water content, and this method minimizes total energy requirements, enhancing the economic viability of the product [24].

Considering the advantages of PEF, key factors influencing the successful disruption of microalgae cells include the electric field E and specific energy input W_{sp}, which can be controlled by manipulating the frequency f, duty cycle D, and treatment time **t** [25]. Therefore, this study aims to 1) investigate the influence of the three main variables f, D, and t on algal oil yield and 2) develop a model for these variables in the context of the PEF extraction process.

II MATERIALS AND METHOD

2.1 Microalgae

The microalgae species used in this study was Tetraselmis sp., cultivated in the Biotechnology Laboratory within the Biological Department of the Faculty of Biology, Universitas Gadjah Mada, Indonesia. For PEF data sampling, a 40 mL microalgae suspension was applied for each experimental process.

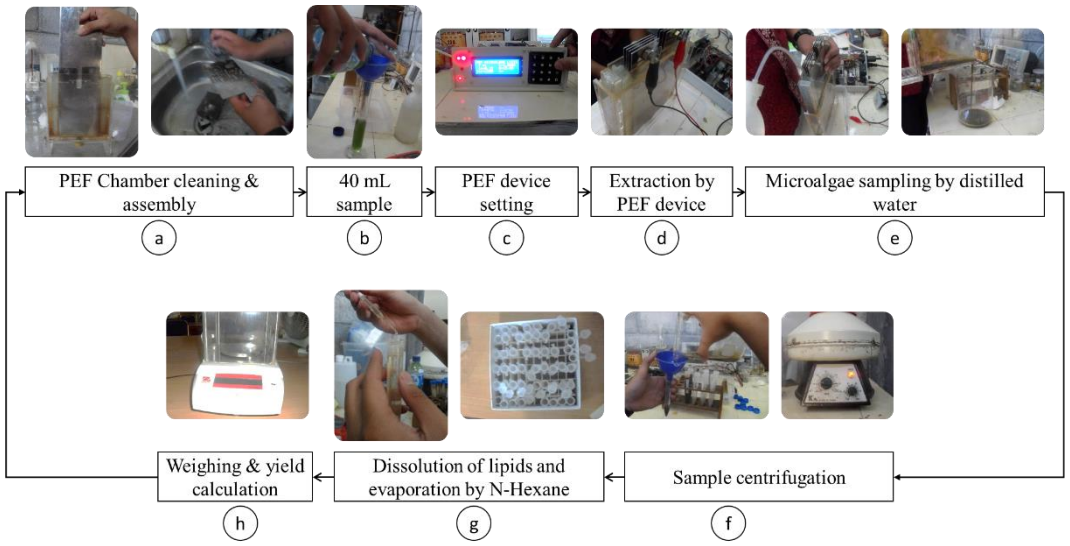


Figure 1. Experimental procedures conducted for the algal oil extraction from Tetraselmis sp. using PEF

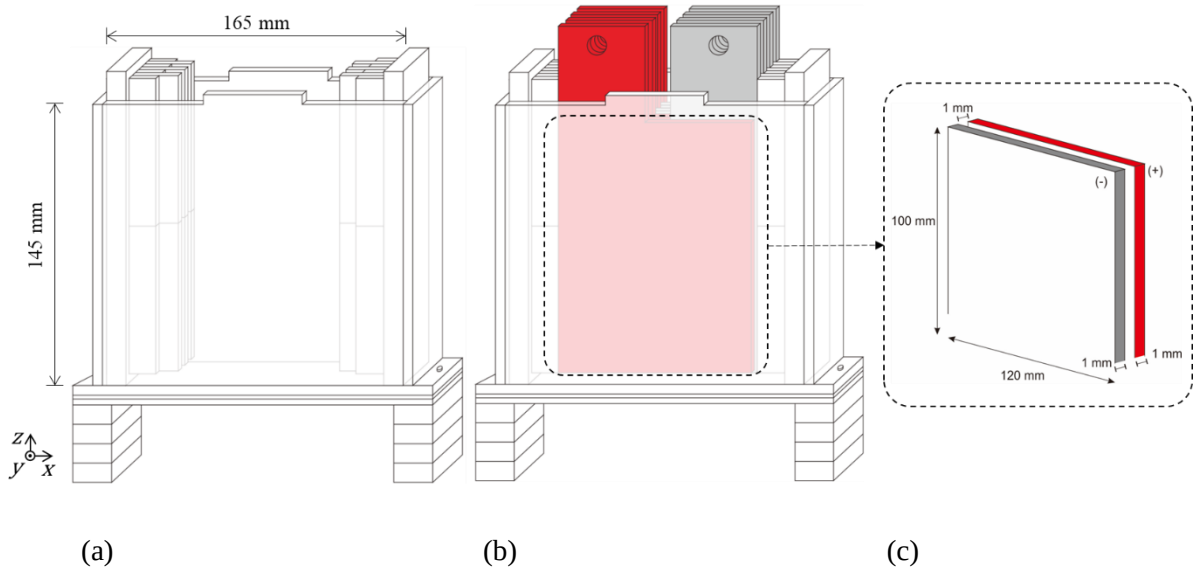


Figure 2. The design of PEF chamber, a) the chamber base, b) stainless-steel plates installation, c) the stainless-steel plates size and gap

Figure 1 shows the experimental procedures carried out during the algal oil extraction from Tetraselmis sp. using PEF. The procedures included: a) PEF chamber cleaning and assembly, b) 40 mL sample preparation, c) PEF device setting, d) Extraction process by PEF device [26], e) Microalgae sampling with distilled water [27], f) Sample centrifugation [28], g) Dissolution of extracted lipids and evaporation using n-hexane [29], and h) Weighing and yield calculation.

2.2 Pulsed electric field (PEF) extraction

In this study, microalgae suspensions were placed in a previously designed treatment chamber. Figure 2 shows the design of PEF chamber used in the experiment, comprising: a) the chamber base, b) stainless-steel plate installation, and c) stainless-steel plate size and gap [26]. PEF chamber was constructed for batch processing, accommodating a maximum volume of 100 mL for each sample. Additionally, it was manufactured in the Eco Mini Plant within the Faculty of Engineering at Universitas Gadjah Mada, Indonesia. According to Figure 2 (a), (b), and (c), the chamber consisted of eight stainless steel plates, each measuring 100x120 mm in size and 1 mm in thickness, placed on a 165x145x42 mm acrylic base.

PEF operates through an electric field **E** formed between two parallel metal plates, depending on the potential difference applied at a specific distance, and this relationship is expressed as follows:

$$|\mathbf{E}| = \frac{\Delta V}{d} \quad (1)$$

Where $|\mathbf{E}|$ represents the electric field magnitude [volt/m], ΔV is the applied potential difference [volt], and d is the distance between the two parallel metal plates [m].

The distance between each plate was set to 1 mm and maintained with a glass material for isolation. A fixed voltage source V_s=168 V (DC) was used, generating an electric field of 1.68 kV/cm, as calculated with Eq. (1). To control PEF voltage pulse, a pulse width modulation (PWM) was determined by adjusting the V_s ratio applied to PEF chamber, expressed as follows:

$$D = \frac{t_{V_s^{on}}}{t_{V_s^{on}} + t_{V_s^{off}}} [100\%] \quad (2)$$

Where D represents the duty cycle [%], $t_{V_s^{on}}$ is the duration of V_s during the turned-on period, and $t_{V_s^{off}}$ is the duration of V_s during the turned-off period. The relationship between D and ΔV is expressed as:

$$\Delta V = DV_s \quad (3)$$

In this study, the influence of D, f, and t on the yield of microalgae extraction using PEF method was explored through variables and parameters settings outlined in Table 1.

Table 1. The PEF extraction variables and parameters settings in the experiments.

Variables	Number of experiment variation		
	Step I	Step II	Step III
Duty cycle D [%]	3, 6, 9, 12, 15	Best value of step I	Best value of step I
Frequency f [Hz]	1 (fixed)	1, 2, 3, 4, 5	Best value of step II
Treatment time t [min.]	20 (fixed)	20 (fixed)	5, 10, 15, 20, 25

According to Table 1, PEF extraction experiments were conducted in three steps. Step I comprised setting the f and t as a fixed value while varying D to investigate its impact. In Step II, t was remained constant, D was selected based on the best result from Step I, and f was changed. In Step III, both D and f were fixed and selected based on the results of Step I and Step II, while t was altered. The purpose of varying D, f, and t was to identify the best combination of variables for achieving maximum extraction results.

2.3 Lipid yield calculation and mathematical model

During the experiment, the lipid yield percentage was determined by calculating the weight ratio of microalgae oil product [30] using the following formula:

$$Y = W_o / W_m \quad (4)$$

Where W_o is the weight of the oil product [g], W_m is the weight of microalgae samples [g], and Y represents the oil yield [%]. Besides, a conventional solvent extraction model was mathematically created for the results of PEF extraction yield. This kinetic model was proposed [30] to approximate the lipid mass transfer that occurred during the extraction process, using the following equation:

$$\frac{1}{\tau}\frac{dY'}{dt} = K - Y \quad (5)$$

Where Y' is the lipid yield (extracted lipid per 100 grams of dry microalgae), K denotes the maximum yield (extracted lipid per 100 grams of dry microalgae) obtained through PEF, t represents extraction time (minute), and τ is the time constant (1/minute). Through integration and by considering the non-linearity of each parameter [31], Y' in Eq. (5) is subsequently expressed as:

$$Y_t' = K(1 - e^{-\tau/t}) \quad (6)$$

Where K is the maximum yield, once its value is high, the extracted lipid becomes significantly high, indicating that the extraction process is qualitatively effective. In the case of relatively small K, the extracted lipids appear to be small. Besides, a higher value of τ means the extraction process runs slowly and a long time is required to achieve maximum yield. A lower τ value means only a short time is needed to attain maximum yield.

In this study, the values of K and τ were the functions of the investigated variables, such as D and f. Assuming that PEF operational variables strongly influence K while τ is more influenced by PEF chamber dimensional factors, K can be approximated as follows:

$$K = K_0 \eta_D \eta_f \quad (7)$$

Where η_D represents the duty cycle efficiency factor, and η_f is the frequency efficiency factor.

III RESULT AND DISCUSSION

3.1. Experimental results

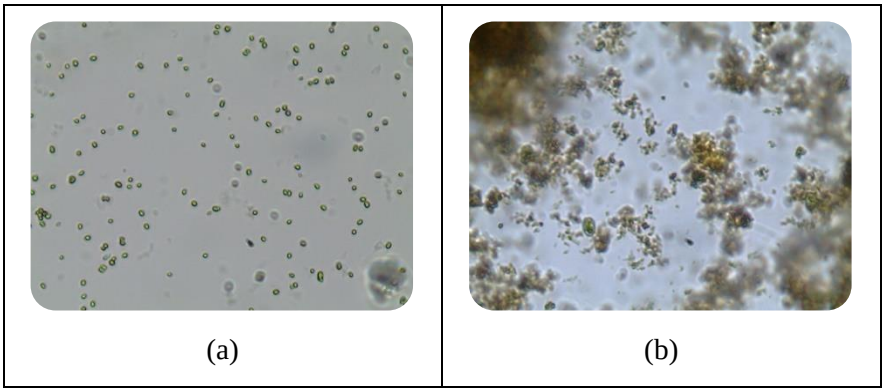
3.1.1. Visual analysis of microalgae samples before and after PEF extraction

Figure 3 shows a visual representation of microalgae samples (a) before and (b) after PEF extraction. Under microscopic observation at 40x magnification, obvious structural and distribution changes were evident in the macroalgae cells. The structural integrity of the cells was compromised, and dead cells were agglomerated post-extraction.

3.3.2 Yield results

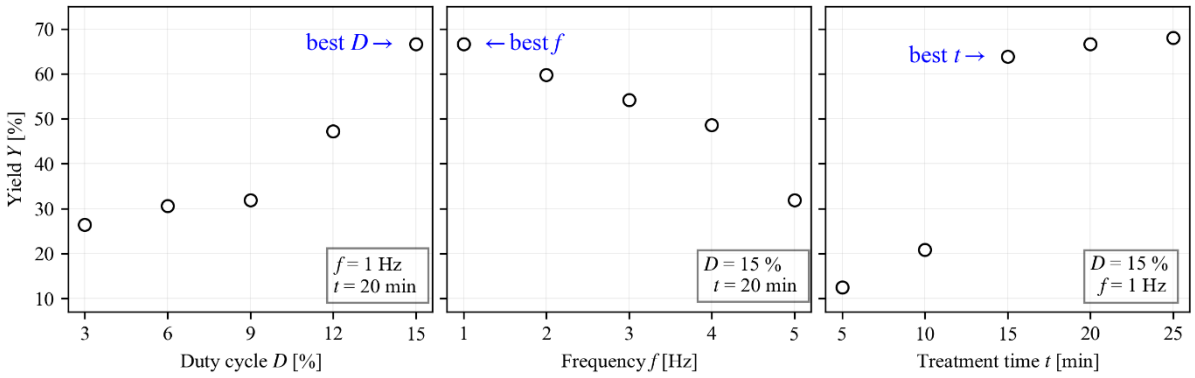
Figure 4 presents the experimental results of *Tetraselmis sp.* extraction through PEF treatment based on the parameters settings in Table 1. The results were displayed in three segments as follows: (a) Step I: different D with fixed f and t (b) Step II: varying f value in the presence of constant D and t, (c) Step III: different t with fixed D and f. Y was calculated using Eq. (4), representing the percentage lipid yield of *Tetraselmis sp.* extracted in a previously designed PEF Chamber [26].

In general, in Step I (Figure 4(a)), increasing D led to significant increment in Y. However, in Step II (Figure 4(b)), the elevation of f initiated the decrement in Y. In Step III (Figure 4(c)), longer t led to an increased Y. A saturation condition was observed at t =15 min, after which there was no significant change in Y. Across each step in Figure 4, the optimal conditions for each variable were observed at D = 15%, f = 1 Hz, and t = 15 min.



(a) (b)

Figure 3. A visual representation of the samples under the microscopic observation (40x), (a) before and (b) after PEF extraction



(a) (b) (c)

Figure 4. Experimental results of Tetraselmis sp. extraction through PEF treatment based on the parameters settings shown in Table 1 as follows: (a) Step I: different D with fixed f and t (b) Step II: varying f values in the presence of constant D and t (c) Step III: different t with fixed D and f [26]

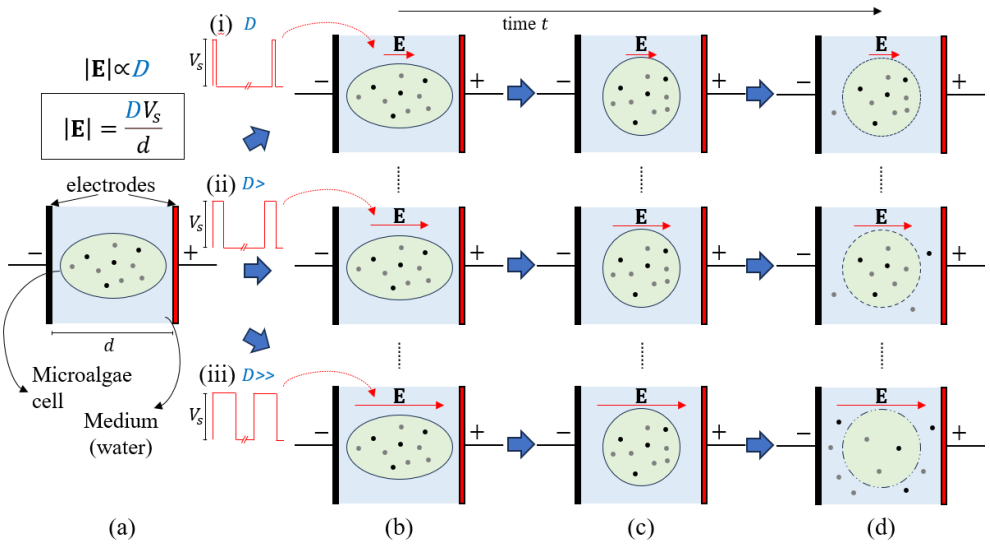


Figure 5. Representation of various stages of microalgae cell membrane electroporation, featuring (a) zero potential (b) osmotic imbalance, (c) swelling, and (d) membrane ruptures extracted by PEF (modified from [32], [33]) under different duty cycle conditions, including (i) D, (ii) higher D (D>), and (iii) higher D> (D>>)

IV DISCUSSION

4.1 Effect of duty cycle on PEF extraction yield

Figure 5 shows a representation of various stages of microalgae cell membrane electroporation, featuring (a) zero potential (b) osmotic imbalance, (c) swelling, and (d) membrane ruptures extracted by PEF (modified from [32], [33]) under different duty cycle conditions, including (i) D, (ii) higher D ($D>$), and (iii) higher $D>$ ($D>>$). During the experiment, the application of PEF to microalgae samples induced an osmotic imbalance (Figure 5b) and cell swelling (Figure 5c). Subsequently, as the electric field magnitude $|\mathbf{E}|$ became stronger, it caused the cell membranes to rupture due to an increasing number of pores. By maintaining $|\mathbf{E}|$ above a certain threshold value, the cell wall experienced electroporation, leading to the extraction of internal cellular contents [33]. As explained in Eqs. (1)~(3), D had a proportional relationship to $|\mathbf{E}|$, with stronger $|\mathbf{E}|$ corresponding to higher D values. Based on Figure 5, the application of higher D starting from (i) D, to (ii) $D >$ and (iii) $D >>$, initiated significantly greater electroporation. As indicated in Figure 4a, D strongly influenced the extraction process, with its higher values leading to an exponential increase in Y, and this relationship could be expressed as follows:

$$\eta_D = e^{\alpha D} \tag{8}$$

Where α represents the duty cycle percentage factor model for PEF extraction. Since the highest Y was achieved at D = 15 % in Step I, as shown in Figure 4a, this parameter value became the benchmark for Step II.

4.2. Effect of frequency on PEF extraction yield

In Step II, the highest Y value was obtained at an f value of 1 Hz, with a fixed D of 15%. In this setting, 15% of the total DC voltage source V_s was supplied to the microalgae samples every 1 second. The results from Step II showed that higher f led to smaller Y values. This observation was attributed to the faster PEF switching time associated with higher f , which inhibited complete discharge of the capacitor component in the PEF rectifier. The microalgae cell membrane was modelled as a capacitor with a dielectric medium subjected to a potential voltage ΔV [34]. From this analogy, it could be inferred that the frequency efficiency factor η_f in Eq.(7) was inversely proportional to the frequency, expressed as follows:

$$\eta_f = e^{-(\beta f)} \quad (9)$$

Where β represents the frequency efficiency constant model for PEF extraction. As indicated in Figure 4(c), changes in Y over time t seemed to have the yield growth curves factor L . Consequently, a Logistic equation was considered to model this behavior [35]. By substituting Eq. (9) into Eq. (7) in accordance with the Logistic equation, the modelled yield was calculated as:

$$Y'_{D,f,t} = K_0 + \frac{L}{1+e^{\left(\alpha D-\beta f+\frac{\tau}{t}\right)}} \quad (10)$$

To determine the best values of K_0, L, α, β, and τ, each parameter was tested by comparing the Y' values in Eq. (10) with the Y obtained experimentally through Eq. (4). Data fitting was achieved by maximizing the coefficient of determination (R^2) for each $Y'_{D,f,t}$ compared to the $Y_{D,f,t}$ data generated through numerical computation. The R^2 is commonly calculated using the following equation:

$$R_i^2 = \frac{N_i \sum Y_i Y_i' - \sum Y_i \sum Y_i'}{\sqrt{\left[N_i \sum Y_i^2 - (\sum Y_i)^2\right]\left[N \sum Y_i'^2 - \left(\sum Y_i'\right)^2\right]}} \quad (11)$$

Where i represents the chosen independent variable of D, f, or t, and N_i is the number of experimental data points derived from each i. Consequently, based on the R_i^2 scores calculated using a numeric computing platform software, all the constant values for Eq. (10) were determined as: K_0 = 70.7346, L= -52.1521, α = 0.4993, β = 0.7798, and τ = -87.5942. Finally, Figure 6 shows the fitting results based on the calculations obtained from Eqs. (10) and (11).

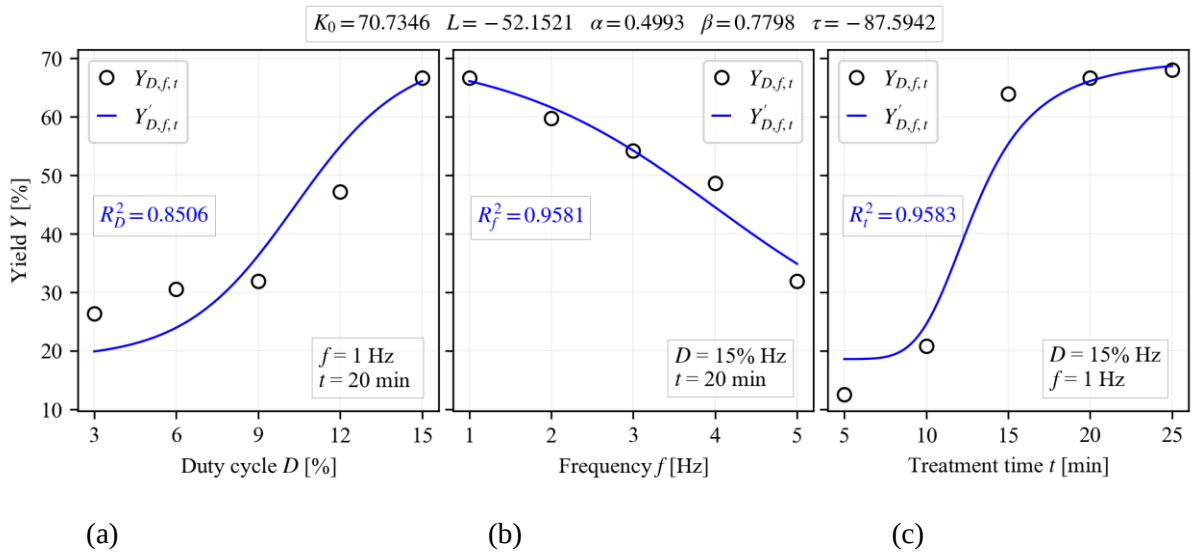


Figure 6. The fitting results of $Y'_{D,f,t}$ and R_i^2 obtained at K_0, L, α, β, and τ in (a) Step I: different D with fixed f and t, (b) Step II: varying f in the presence of constant D and t, (c) Step III: different t with fixed D and f

Figure 6 presents the fitting results of $Y'_{D,f,t}$ and R_i^2 obtained at K_0, L, α, β, and τ in (a) Step I: different D with fixed f and t, (b) Step II: varying f values in the presence of constant D and t, and (c) Step III: different t with fixed D and f. In this context, the highest R_i^2 was obtained at R_t^2=0.9583, followed by R_f^2=0.9581, with the lowest being observed at R_D^2=0.8506. The average of all coefficients of determination$\overline{R_i^2}$ was calculated as $\overline{R_i^2}$ =0.9223. Moreover, each R_i^2 value between PEF method and the gathered experimental data was relatively high, exceeding 0.85 with an average above 0.92. The high R_i^2 showed a strong relationship between each variable and its paired parameter among the experimental setup indicated in Table 1, further validating the developed PEF extraction model as presented in Eqs. (5)~(10).

Through the application of the correct sample and appropriate $|\mathbf{E}|$, D, and f values, PEF was indicated to significantly influence the extraction process. Furthermore, it was inferred from Figures 4c and 6c that longer t led to higher Y. The increase in Y was found to become less significant at t >15 mins. This less significant effect was attributed to the complete damage of *Tetraselmis sp.* cell membranes after 15 mins, leaving only a minimal amount of undamaged microalgae. To enhance Y over time t and advance PEF extraction process, the implementation of a continuous PEF device was recommended. The entire experimental procedure presented in Figure 1 was conducted in a batch process. Specifically, the weighing process depicted in Figure 1 h could be improved through *in-situ* measurement techniques [36]. For instance, an *in-situ* electrical resistance measurement [37] within the time domain [38] could offer a real-time estimation of extraction results (yields), potentially improving the total efficiency of the process.

V. CONCLUSION

In conclusion, this study thoroughly examined the influence of duty cycle D, frequency f, and treatment time t on microalgae extraction yields using PEF method, while maintaining a constant electric field E of 1.68 kV/cm. The results were modelled to elucidate the effect of each variable on the extraction process. The model parameters were determined as follows: maximum yield (grams of extracted lipids per 100 grams of microalgae) K_0 = 70.7346, yield growth curves factor L=-52.1521, duty cycle percentage factor α = 0.4993, frequency efficiency constant β = 0.7798, and time constant τ = -87.5942. The proposed model exhibited a relatively high coefficient of determination (R_i^2), where R_t^2=0.9583, R_f^2=0.9581, and R_D^2=0.8506, with an average $\overline{R_i^2}$ of 0.9223.

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