ALGAE LYSIS
WITH PULSED ELECTRIC FIELDS

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ABSTRACT

Algae Lysis with Pulsed Electric Fields

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With growing interest in alternative fuels, algae lipid harvesting is seen as a possible source of biofuel. Algae species under consideration include *Chlorella vulgaris*, *Chlamydomonas reinhardtii* and *Dunaliella salina* due to lipid contents as high as 30% to 56% of their dry weight (depending on growth conditions) and availability [5], [6]. In order to harvest lipids from algae, the cells must first be lysed.

Lysing is achieved by breaking the algal cell wall or membrane to separate oil from the rest of the algae biomass. Current lysing procedures use enzymes, pressure homogenization, and/or sonication to lyse cells; however, these methods are costly and complicate oil extraction [9], [10].

This project examines a novel method of cell lysis through pulsed electric field (PEF) application that enables cost-effective extraction methods relative to current enzyme and sonication techniques.

A theoretical model for cell membrane potential in the presence of electric field was developed, and PEF chambers were manufactured on microscope slides to enable microscope viewing and cell lysis recording during PEF application. Additionally, larger static chambers were created for testing higher volumes of algal solution. Electric field characteristics, such as pulse width, pulse number and magnitude, sufficient for lysis of *Dunaliella salina* and *Chlorella vulgaris* were found.

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Chapter 1: Biofuel Applications of Algae and Cell Models for PEF Lysis

The following sections describe the viability of algae based biodiesel, current processing methods, and the theory behind PEF induced lysis.

1.1 Algae Biodiesel Viability Overview

There has been extensive research in algae lipids as a potential feedstock source for biodiesel production. Algae biofuel has several advantages over plant crops including the ability to accumulate large amounts of triacylglycerides, a major biodiesel production feedstock, and the ability to grow in low quality land unsuitable for food crops [6]. Table 1.1 shows recorded lipid contents of several algal species. [7]

Species	Lipid Content (%dry weight)
<i>Scenedesmus obliquus</i>	11–22% / 35–55%
<i>Scenedesmus dimorphus</i>	6–7% / 16–40%
<i>Chlorella vulgaris</i>	14–40% / 56%
<i>Chlorella emersonii</i> **	63%**
<i>Chlorella protothecoides</i>	23% / 55%
<i>Chlorella sorokiana</i>	22%
<i>Chlorella minutissima</i>	57%
<i>Dunaliella salina</i>	14–20%
<i>Neochloris oleoabundans</i> **	35–65%**

Table 1.1 Algae Species and Lipid Content [7]

While Table 1.1 is not exhaustive of algae species suitable for biofuel research and does not describe possible lipid contents for all growth conditions, it does show that lipid contents are comparable to other biodiesel feedstock, Table 1.2.

Crop	Oil Content per ton of biomass (%dry weight)
Oilseed rape	40-44% (of seed)
Soybean	20% (of seed)
<i>Jatropha</i>	30% (of seed)

Table 1.2 Oil Content for Plant Crops [6]

Additionally, algae species should yield more liters per hectare (L/10000m² or L/ha) of oil than other plant based crops used for biodiesel production, Table 1.3.

Crop	Oil yield (L/ha)	Percent of existing US crop area required to supply 50% of US transport fuel requirements
Corn	172	846%
Soybean	446	326%
Canola	1190	122%
<i>Jatropha</i>	1892	77%
Coconut	2689	54%
Oil Palm	5950	24%
Microalgae A	136,900	1.1%
Microalgae B	58,700	2.5%

Microalgae A: 70% oil (by weight) in biomass

Microalgae B: 30% oil (by weight) in biomass

Table 1.3: Comparison of Biodiesel Sources [8]

Table 1.3 shows that relative to necessary crop area, algae is possibly the only feasible crop to displace fossil fuel. Unlike other crops, many algae species can double their biomass within 24 hours [8].

However, there are several challenges to harvesting algae and separating algal lipids from biomass in its growth media for acceptable algal biodiesel energy efficiency and cost effectiveness. This thesis considers algae cell wall lysis to separate lipids from the enclosed cell. An overview of algal biodiesel processing is discussed.

1.2 General Process for Converting Algae to Biodiesel

To process algae from its growth media into biodiesel, several processes must be performed. In general, the process includes algae cultivation, harvesting or collection from growth media, processing that releases and separates algae oil from the algal biomass, and oil treatment, such as transesterification, to produce biodiesel [6]. Each process must be optimized for algae to be a viable biodiesel feedstock.

In the growth process, manipulating available light and nutrients available to the culture optimizes triacylglyceride production; however, this compromises the growth of many algal species [6].

Multiple methods exist for collecting and concentrating algae from its growth media including centrifugation, flotation, and filtration. Centrifugation uses centrifugal force to separate algae from its media, flotation uses micro-bubbles to float algae to the top of the growth media for separation, and filtration uses a filter to collect algae. However, the effectiveness of these procedures largely depends on algae cell size and solution salinity. There are also several drawbacks for each process. Centrifugation apparatus cost is proportional to increased scale. Flocculation (the formation of colloidal clusters) occurs with flotation micro-bubbles and may

contaminate the collected algae. Filtration efficiency is largely dependent on filter membrane and filtration method [10].

Current commercially viable processes for releasing lipids from the cell membrane include enzymatic and mechanical lysis; both discussed in section 1.3 below.

Once cells are lysed, the oil must be recovered. Typically, solvents such as hexane extract oil from the solution. However, this denatures the cellular material; decontamination becomes difficult [10]. A classic example of denaturation is the hardening of an egg during cooking, causing a loss of solubility. A centrifuge can be used to separate oil from the biomass if the solution does not contain emulsified proteins, which can be created from mechanical lysis processes.

The final step is lipid transesterification into biodiesel [7]. All aforementioned processes must be combined into a coherent system for feasible algae based biodiesel production. However, this thesis focuses on the separation of algal lipids from the enclosed cell membrane.

1.3 Current Algae Lysis Methods and Proposed PEF Method

This section briefly describes predominant commercial algae lysis methods for algae harvesting and the method proposed in this thesis. It should be noted that several other methods exist, such as heating; however, the techniques listed below are the most feasible in terms of energy efficiency [10].

1.3.1 Enzymatic Lysis

Enzymatic lysis is the disruption of a cell's wall by enzymes. The enzyme causes algal cell wall breakdown and release of the cells' enclosed material. However, enzymes are expensive, require additional extraction from the treated algal biomass, and can complicate oil separation [9], [10].

1.3.2 Mechanical Lysis

Several mechanical lysis methods have been developed, such as pressure homogenization and sonication. Pressure homogenization involves pressurizing algal solutions through an orifice to cause cell wall disruption. Sonication uses high frequency pressure waves to disrupt the cell wall. However, both of these processes significantly heat the process fluid, which can emulsify and denature soluble proteins, complicating oil extraction[10].

1.3.3 PEF Lysis

PEF (pulsed electric field) induced lysis, the method proposed in this paper, is achieved by subjecting algae to pulsed electric fields. This non-invasive method seeks to avoid algal solution contamination while avoiding excessive heating to enable centrifugal oil extraction from the treated algal solution. The following sections outline PEF cell lysis theory .

1.4 Membrane Potential in the Presence of Electric Fields

This section reviews cell potential models in the presence of electric fields and cell wall breakdown during lysing procedures.

Figure 1.1: Blood Cell Model [1]

A blood cell model [1] assumes a spherical shape with inner and outer radii a and b , respectively, and membrane thickness d . σ_1 , σ_2 , σ_3 and φ_1 , φ_2 , φ_3 denote conductivities (material characteristics) and potentials (related to the applied electric field) in the cell interior, cell membrane, and external medium regions, respectively. Applying boundary conditions for $\phi(r)$, r = radial parameter:

$$\begin{aligned}\varphi_1(a) &= \varphi_2(a) \\ \varphi_2(b) &= \varphi_3(b) \\ \sigma_1 \frac{d\varphi_1}{dr} &= \sigma_2 \frac{d\varphi_2}{dr} \text{ at } r = a \\ \sigma_2 \frac{d\varphi_2}{dr} &= \sigma_3 \frac{d\varphi_3}{dr} \text{ at } r = b\end{aligned}\tag{1.1}$$

These relations are derived from tangential electric field boundary conditions; refer to Appendix A.

Zimmerman derives the potential difference across the cell membrane, transmembrane potential, from (1.1)

$$V_m = \frac{\frac{3}{2+\beta} E_o \left[a - b + \frac{1-\alpha}{2+\alpha} a^3 \left(\frac{1}{a^2} - \frac{1}{b^2} \right) \right] \cos \vartheta}{1 + 2 \left(\frac{1-\beta}{2+\beta} \right) \left(\frac{1-\alpha}{2+\alpha} \right) \left(\frac{a}{b} \right)^3}\tag{1.2}$$

where $\alpha = \sigma_1/\sigma_2$ and $\beta = \sigma_2/\sigma_3$. Zimmerman considers $\sigma_1 \approx \sigma_3 \gg \sigma_2$ for blood cells, where $\sigma_1 = 0.005$ (S/cm), $\sigma_3 = 0.01$ (S/cm), and $\sigma_2 = 10^{-7}$ S/cm. Zimmerman's [1] assumption of $\sigma_1 \approx \sigma_3$ may be due to the ionic balance of the exterior cell medium and the cell's cytoplasm. The membrane is also assumed to exhibit lower conductance compared to the cytoplasm and medium due to lipid content, as lipids (such as fats) are insulators. These two assumptions are

applied to algal cells using the above relations. Therefore, $\alpha \rightarrow \infty$ and $\beta \rightarrow 0$, simplifying equation (1.2)

$$V_m = \frac{\frac{3}{2}E_o \left[a - b - a^3 \left(\frac{1}{a^2} - \frac{1}{b^2} \right) \right] \cos \vartheta}{1 - 2 \left(\frac{1}{2} \right) \left(\frac{a}{b} \right)^3} \quad (1.3)$$

$$V_m = \frac{\frac{3}{2}E_o \left[-b + \left(\frac{a^3}{b^2} \right) \right] \cos \vartheta}{1 - \left(\frac{a}{b} \right)^3}$$

$$V_m = -1.5E_o b \cos \vartheta \quad (1.4)$$

A more general solution by Pohl [2] for a two-layered cell yields similar expressions for the transmembrane potential using Zimmerman's assumptions on cell conductivity. In Appendix A, the expression given by Zimmerman in (1.4) is verified by deriving the transmembrane potential for a two layered cell. This analysis shows that multiple layered cells, assuming $\sigma_{outermost\ cell\ layer} \ll \sigma_{cell\ medium}$ have the same membrane potential as cells with single layers.

Figure 1.2: Two Layered Cell Model [2]

1.5 Cell Membrane Electrical Breakdown

Figure 1.3: Cell Membrane Electrical Breakdown [3]

- (A) The membrane is modeled as a capacitor with a dielectric medium. The potential across the membrane without the presence of an electric field (i.e. resting potential) is 10mV.
- (B) The presence of an external electric field causes an increase in membrane potential (see Eq. (1.4)). This increase causes cell membrane compression.
- (C) Membrane breaks down at the critical voltage V_c , where pores are formed in the cell membrane and are filled with solution outside the cell membrane. Breakdown is

reversible if the size and number of pores is not sufficient to maintain breakdown. Pore location is a result of electric field intensity, where the portion of the cell membrane exactly tangential to the applied field forms the highest potential (see equation (1.4)).

- (D) For longer exposure times to fields inducing critical membrane voltage and for fields inducing transmembrane potentials much greater than the critical potential, breakdown will become irreversible due to the size and number of pores formed [3].

The above figure describes the formation of cell membrane pores caused by cell membrane electrical breakdown. Cell membrane “reversible breakdown” occurs at the critical membrane potential V_c , where the cell membrane forms pores but is able to reform or 'reverse' breakdown after removing the external field. “Irreversible breakdown” occurs when the electric field produces a transmembrane potential much greater than the critical voltage, creating many large 'irreversible' pores. According to Zimmermann et al. [3],

A number of models [for electrical breakdown] have been proposed, among them one which postulates that electrical breakdown leads to local instabilities in the membrane by way of electromechanical compression and electrical field-induced tension, so that pores are able to form... Although the pore model is certainly a very lucid one, we have to point out that the molecular interpretation of electrical breakdown in terms of pores is not proven.

It is concluded that the exact mechanisms for electrical breakdown and pore formation are still the subject of research and focus is placed on determining threshold voltages, pulsewidth, repetition rates, etc for algal cell lysis.

Chapter 2: Microscope View Slide PEF Chamber

A disadvantage of using copper electrodes with water contaminated with minerals and salts is electrolysis (chemical decomposition produced by passing current through an electrolyte). When charged, copper electrodes are submerged in an electrolyte, or water with salt ions such as NaCl. The copper reacts with electrolyte ions causing copper ions to leech into the solution. The current drawn from the source is therefore not an indication of charge through the sample, but the rate of electrons exchanged with electrodes and electrolyte ions. To ensure full potential application across the algal sample, silver/silver chloride electrode test chambers were created, which have the advantage of freely passing electrons, as opposed to ions through the electrode-electrolyte barrier. This allows DC voltage application without electrode interaction with the electrolyte. The silver/silver chloride electrodes were created by cleaning 0.25mm silver plates with pre-soldered 22 gauge magnet wire leads in reagent alcohol (90% Ethanol, 5% Methanol, 5% Isopropyl Alcohol), and soaking in bleach for approximately 30 minutes. The bleach creates a silver chloride layer on the silver surface, visible as black/purple tarnish.

2.1 Laser Etched Acrylic Microscope View Slides

To view algae during PEF application, acrylic microscope slides were created, see Figure 2.1. A 1mm x 1mm x 1mm etched spacing was milled for fluid algae sample placement between silver/silver chloride electrodes.

Figure 2.1: Cubic Millimeter Sample Chamber

Algae samples were placed in these slides and viewed under a microscope. It was found that the laser etching process creates a porous surface, which obstructs sample visibility. Additionally, the microscope's focal plane is unable to focus on algal cells because the 1mm sample depth allows multiple algae layers and movement out of the focal plane.

It was determined that the vertical sample depth should be minimized to less than one millimeter and the transparent sample surface be preserved on the next iteration of manufactured slides.

2.2 Revised Silver/Silver Chloride Glass Microscope View Slides

The sample size features 1mm electrode spacing, 1mm sample length, and 0.25mm sample depth. Non-conductive cyanoacrylate adhesive (Super Glue) was used to attach the electrodes and create the sample area.

Figure 2.2: 1mm Spacing Silver Chloride Electrodes

Below is the microscope slide from Fig. 2.2 with centrifugally concentrated *Chlamydomonas* in the electrode sample area.

Figure 2.3: Chlamydomonas Algae Between Silver/Silver Chloride Electrodes

2.3 Initial Test with Silver/Silver Chloride Slide and Dielectrophoretic Effect

A video of the *Chlamydomonas* algae was recorded while a 5V potential was applied across the sample for approximately 15s. The video shows a net movement of algae to the positive electrode. The results are shown in Fig. 2.4 below.

Figure 2.4: Chlamydomonas Algae After 15s Exposure to 5V DC, $|E| = 50 \text{ V/m}$

The observed movement is possibly due to dielectrophoretic force applied to the algae cell as a result of non-uniform electric field fringing at the upper and lower conducting layers of the algae sample. Dielectrophoresis is a phenomenon where a dielectric particle, such as algae cells, is subjected to an electric field induced force with what result? Lysis? Movement? [2].

With a sample depth of 0.25mm and algae cell diameters of approximately $10\mu\text{m}$, several layers

of algae are outside the microscope's focal plane, which made individual cell viewing difficult in subsequent tests.

2.4 Gold Leaf Electrodes

To reduce sample depth, electrodes were manufactured using 23k gold leaf with 1 μ m thickness, and aerosol adhesive. Gold was chosen for its chemically neutral properties, since it does not readily interact with electrolytes in the presence of direct current. The sample area was etched away with a razor blade and cleaned with reagent alcohol, see Fig. 2.5.

Figure 2.5: Gold Leaf Coated Glass Microscope Slide

The microscope revealed that the adhesive insulates the gold's interface from the sample, prohibiting conduction. The fragility of the gold leaf and inconsistent conduction prohibits this method of slide construction.

2.5 Microscope Cover Slip With Silver/Silver Chloride Electrodes

Silver/silver chloride electrodes with 2cm spacing were manufactured and tested. This enables the use of coverslips to limit sample depth, while allowing conduction across the sample. At 20V DC ($|E| = 10\text{V/cm}$, 2cm spacing), no effect was observed in the algae sample. This is likely due to insufficient electric field strength to cause observable dielectrophoresis.

2.6 Microscope PEF Chamber Performance Comparisons and Conclusion

The silver/silver chloride electrode PEF chambers were the easiest to construct, yield optimal electrode spacing to observe dielectrophoresis, and were the most robust. For these reasons, they were selected for use in subsequent algae cell lysis tests.

Chapter 3: Experiments in PEF Characteristics for Lysis

This chapter documents the experiments through which PEF characteristics such as amplitude, duration, and repetition are optimized.

3.1 Single Pulse Treatment of *Chlamydomonas*

A one millimeter spaced silver/silver chloride electrode chamber was used to apply 15V pulses to centrifugally concentrated *Chlamydomonas* in a regular growth solution of water and Shultz plant food. The applied voltage corresponds to 150V/cm electric field between the electrodes. The sample dimensions were 0.25mm depth with 5mm length, yielding a 1.25uL sample volume with 1mm spaced electrodes.

Single 1us, 1ms, and 1s pulses were applied to separate *Chlamydomonas* samples. No effect was observed for 1us pulse. Figure 3.1 shows the *Chlamydomonas* before and after 1ms pulse treatment. Figure 3.1 shows a net movement of algae after pulse application to the bottom positive electrode, where the top electrode is at ground potential, which is predicted by Pohl [1] when applying non-uniform electric field. The non-uniform field may be a result of the inherent inhomogeneity of the algae sample.

Figure 3.1: Before and After Pictures of Chlamydomonas Sample for 1ms, 150V/cm Pulse (200x Magnification)

Figure 3.2: Before and After Pictures of *Chlamydomonas* Sample for 1s, 150V/cm Pulse (200x Magnification)

- (A) Algae before treatment
- (B) 250ms: Early bubble formation
- (C) 750ms: As larger bubbles form, more algae move toward bottom positive electrode
- (D) Algae after 1s pulse treatment: Algae collect at bottom electrode with large bubble formation from heating.

Figure 3.2 shows a photo sequence during application of the 1s, 150V/cm pulse. The pulse caused either significant heating or an observable electrochemical reaction with the electrode and algae medium as indicated by the formation of bubbles. The longer exposure also caused more algae to collect at the positive electrode compared to the 1ms pulse. This is due to longer exposure to electric field, and therefore dielectrophoretic force, which results from non-uniform fields. Non-uniform fields may be due to the inhomogeneity of algal sample. Additionally, movement to the positive electrode may be due to bubble formation at negative electrode.

Experiment Conclusions

Pulse lengths of 1s and 1ms have observable cell movement predicted by dielectrophoretic force. However, 1s pulse widths result in significant bubble formation. In both cases, algae near the positive electrode ceased movement, but many mobile cells were still intact. This indicates damage to some cells.

3.2 Repetitive Pulse Treatment of *Chlamydomonas*

A 1.0mm spaced silver/silver chloride electrode chamber was used to apply 15V pulses to centrifugally concentrated *Chlamydomonas* in a regular growth solution of water and Shultz plant food. The applied voltage develops a 150V/cm electric field between the electrodes. The sample dimensions are 0.25mm depth with 5mm length, yielding a 1.25uL sample volume with 1mm spaced electrodes. Table 1 shows applied pulse signal characteristics.

Pulse Width	Frequency	Duty-Cycle	Approximate Pulses Applied	Treatment Time	Applied Energy
10ms	14.29Hz	14.29%	476	33.3s	19.47 mJ
100ms	1.429Hz	14.29%	43	41.3s	17.59 mJ

Table 3.1: Signal Characteristics for 150V/cm Continuous Pulse Treatment

Two pulse patterns were applied to two separate *Chlamydomonas* samples. The first pulse pattern period is 700ms, with a 100ms pulse with 600ms separation between pulses. This corresponds to a 14.29% duty cycle, pulse width to period ratio, in percent. The first pulse pattern was used to treat the sample for approximately 33.3s Fig. 3.3 shows the oscilloscope-captured pulse pattern. Figure 3.4 below shows pictures of the sample during 100ms pulse pattern application.

Fig 3.3: 14.29% Duty-Cycle, 100ms Pulses

Figure 3.4: 14.29% Duty Cycle 100ms Pulses for 33.3s, 429 pulses (200x magnification)

- (A) Sample prior to pulse pattern application
- (B) 5 sec: Formation of bubbles during treatment time and movement of algae cells to the positive electrode
- (C) 15 sec: Further algae cell grouping and larger bubble formation
- (D) Sample after 30 sec treatment

Figure 3.5: After 30s Treatment with 14.29% Duty Cycle 100ms Pulse Width, 43 Pulses (100x Magnification)

Similar to previous experiments with single pulses, algae collects at the positive electrode (top) and bubbles form at the negative electrode. Since the cells move during pulse treatment, it is difficult to focus on a single cell at sufficient magnification to view lysis. Therefore, samples of post-treated cells were placed in a hemocytometer to view cell integrity.

Figure 3.6: After 30s Treatment with 14.29% Duty-Cycle 100ms Pulse Width, 43 Pulses (1000x Magnification)

Figure 3.6 shows 100ms pulse-width, 43 pulses, 14.3% duty cycle (30sec) treated cells on a hemocytometer. It appears that several cell walls have been porated, which is indicated by "fuzzy" cell boundaries. However, cell organelle and chloroplast release is not apparent.

The second pulse pattern, Figure 3.8, is 10ms pulse-width, 476 pulses, 14.3% duty cycle (32sec). Figure 3.7 below shows the pulse pattern measured on the oscilloscope.

Figure 3.7: 14.29% Duty-Cycle 10ms Pulses for 30s (200x magnification)

- (A) Sample before pulse pattern application
- (B) 5s: Formation of bubbles during treatment time and algae cell collection
- (C) 15s: Further algae cell collection and larger bubble formation
- (D) Sample after 32s treatment

Figure 3.8: 14.29% Duty-Cycle 10ms Pulses

The 10ms pulse pattern exposed sample was placed on a hemocytometer. Similar results were observed between the two experiments; cells appeared porated, but organelle and chloroplast release was not observed. In both cases, cells ceased movement after PEF treatment.

Experiment Conclusions

While it appears that the applied pulses affect cell walls, it is unclear if this is due to heating or electromagnetic effects. In either case, the electric field magnitude does not appear sufficient to collapse the cell wall; however, it may have been sufficient to porate it. If bubbles are formed due to negative electrode heating, additional experiments should use lesser duty-cycle values to minimize heating.

3.3 Continuous Pulse Treatment of *Dunaliella* Samples

Due to a change in lab location and availability of algal samples, tests were continued on *Dunaliella*. *Dunaliella* has no cell wall, which may reduce required lysis energy. Since the medium is salt water, it is also ideal for growth in non-drinkable water. For these reasons, and high oil content, *Dunaliella* is the subject of extensive biofuel research [5], [6], [7].

A 0.25mm spaced silver/silver chloride electrode chamber was used to apply 15 pulses at 15V to centrifugally concentrated *Dunaliella* in a regular growth solution of water and aquarium salt. The applied voltage yields a 600V/cm electric field between the electrodes. The sample dimensions are 0.25mm depth with 5mm length, yielding a 312.5nL sample volume with 0.25mm spaced electrodes. Table 3.2 shows signal characteristics for pulse patterns applied to three different samples.

Test #	Pulse Width	Frequency	Duty-Cycle	Approximate Pulses Applied	Treatment Time	Applied Energy
1	10ms	0.25Hz	0.0025%	8	33.3s	1 mJ
2	10ms	2.00Hz	0.005%	83	41.3s	10.38 mJ
3	50ms	0.50Hz	0.025%	19	37.1s	11.88 mJ

Table 3.2: Signal Characteristics for 600V/cm Repetitive Pulse Treatment

Three tests on *Dunaliella* were completed with varying pulse width, frequency, duty cycle, and duration. Test 1 caused *Dunaliella* to collect on the positive electrode as shown in Figure 3.9 below.

Figure 3.9: Dunaliella After 600V/cm, 10ms Pulse Width, 8 Pulses (400x Magnification)

Figure 3.10: Dunaliella After 600V/cm, 10ms Pulse Width, 8 Pulses (Digital Zoom of Figure 3.9)

It appears that the cell walls have been porated; however, it is not clear whether chloroplast has been released.

Test 2, 83 pulses at 600V/cm, 10ms pulsewidth lysed *Dunaliella* is shown in Figures 3.11 and 3.12 below.

Figure 3.11: *Dunaliella* After 600V/cm, 10ms Pulse Width, 83 Pulses (400x Magnification)

***Figure 3.12: Dunaliella After 600V/cm, 10ms Pulse Width, 83 Pulses
(Figure 3.12 Digital Zoom)***

After Test 2 (600V/cm, 10ms pulse width, 83 pulses), some *Dunaliella* cells' membranes that had been porated by the electric field began to deteriorate. For example, Figure 3.13 shows the fully deteriorated cell membrane with chloroplast and organelles outside the membrane.

Figure 3.13: Dunaliella Lysis After 600V/cm, 10ms Pulse Width, 83 Pulses (400x Magnification Zoomed In)

After Test 3 (600V/cm, 50ms pulse width, 19 pulses), some cells appear to be lysed; however, not all cells have ceased movement. Test 2 shows that pulse repetition rate and the number of pulses affect visible lysis. Figure 3.14 below shows the Test 3 sample after applied pulses.

***Figure 3.14: Dunaliella Lysis After 600V/cm, 50ms Pulse Width, 19 Pulses
(400x Magnification Zoomed In)***

Experiment Conclusions

Since visible lysis was not observed for many cells in Tests 1 and 3, it is concluded that pulse repetition rate may have an effect on producing visible lysis. This may be due to algae sample heating. Therefore, higher magnitude electric fields are used in subsequent experiments.

3.4 Capacitor Discharge in *Dunaliella* Samples

A 0.25mm spaced silver/silver chloride electrode chamber was used to discharge capacitor banks into centrifugally concentrated *Dunaliella* in a regular growth solution of water and aquarium salt. The sample dimensions were 0.25mm depth and electrode spacing with 5mm length, yielding a 312.5nL sample volume. Table 3.3 shows the signal characteristics for the pulse patterns applied to three different samples.

Test	Capacitor Voltage	Max Electric Field	Equivalent Series Capacitance	Approximate Frequency	Approximate Pulses Applied	Approximate Treatment Time	Applied Energy
1	200V	8.0kV/cm	4.4nF	N/A	1	N/A	88 uJ
2	40V	1.6kV/cm	66uF	0.2Hz	10	50s	528 mJ
3	40V	1.6kV/cm	66uF	0.2Hz	5	25s	264 mJ

Table 3.3: Signal Characteristics for Capacitor Discharge

Three tests on *Dunaliella* were completed with charged capacitors. Test 1 involved charging five parallel 100V rated 22nF capacitors to 40V and discharging in series into the sample to create a maximum potential of 200V.

Figure 3.15: Test 1, 22nF Capacitor Bank (one 8.0kV/cm pulse)

Figure 3.16: Dunaliella Lysis After Test 1 (one 8.0kV/cm pulse) (400x Magnification)

Figure 3.17: Dunaliella Lysis After Test 1 (one 8.0kV/cm pulse) (1000x Magnification)

From Figures 3.16 and 3.17, it is clear that lysis is achieved for the short 8kV/cm pulse from the five 22nF series capacitor bank.

Test 2 involves ten 1.6kV/cm pulses. The capacitors were charged and discharged manually; voltage traces are displayed for in Figure 3.18.

Figure 3.18: Discharge Voltage Waveforms for 66uF Capacitor Bank (Test 2: ten 40V pulses) into Dunaliella sample

During Test 2 pulse application, chloroplast was observed leaving the cell membrane after 8 pulses, at 42s of pulse treatment in Figure 3.19 below.

Figure 3.19: Chloroplast Leaving Membrane 42s after ten 1.6kV/cm capacitor discharge pulses (400x magnification)

- (A) 42s: Chloroplast begins to move to upper left side of membrane
- (B) 43s: Half of chloroplast outside membrane
- (C) 43.5s: Chloroplast completely outside of membrane

**Figure 3.20: *Dunaliella* Lysis After Test 2 (ten 1.6kV/cm capacitor discharge pulses)
(400x Magnification)**

***Figure 3.21: Dunaliella Lysis After Test 2 (ten 1.6kV/cm capacitor discharge pulses)
(1000x Magnification)***

Visible lysis was achieved for Test 2 (ten 1.6kV/cm pulses); hence, for Test 3 (five 1.6kV/cm pulses) test conditions were maintained from Test 2 except for the number of pulses (reduced from ten to five). Figure 3.22 shows Test 3 oscilloscope-captured pulse patterns.

Figure 3.22: 40V 66uF Test 3 (five 40V pulses) Capacitor Discharge Waveforms

Figures 3.23 and 3.24 show that lysis was observed for Test 3 conditions.

Figure 3.23: Dunaliella Lysis After Test 3 (five 1.6kV/cm pulses) (400x Magnification)

Figure 3.24: Dunaliella Lysis After Test 3 (five 1.6kV/cm pulses) (1000x Magnification)

Experiment Conclusions

It was shown that a single 8kV/cm electric field pulse was sufficient to lyse *Dunaliella* cells. A decreased field (1.6kV/cm) was then selected for Tests 2 and 3 (ten and five pulses, respectively), which also lysed the cells. It is noted that discharge rates increased for each subsequent pulse; therefore, further investigation is needed to determine if cell lysis affects sample solution conductance. Figure 3.19 from Test 2 shows that porated cell membranes cause internal cell structures to leave the outer membrane.

Overall, the experiment shows that lysis can be achieved by repetitive lower amplitude pulses (1.6kV/cm) or a single pulse of sufficient amplitude (8.0kV/cm). This experiment's test conditions, by inspection, yields more visibly lysed cells than previous experiments; therefore, subsequent experiments quantify the number of lysed cells versus the number of applied pulses.

Chapter 4: Automated Repetitive Capacitor Discharge Circuit

This chapter documents efforts to develop a repetitive capacitor discharge circuit and testing chamber to enable comparative analyses between pulse characteristics applied to algal cells and the number of lysed cells.

4.1 1mL Sample Stainless-Steel Static PEF Chamber

Previous electrode chambers were designed to accommodate microscope viewing of cell lysis ; however, this limited algal sample volumes to 0.3125uL for chambers with 0.25mm spacing. Accurate control of sample volume and 10uL algal solution to the hemocytometer for cell counting after pulse treatment was difficult. To allow sufficient treated sample volume for cell counts, a stainless steel chamber was created using two 127mm x 127mm x 1mm stainless steel plates. Vertical spacing between the plates was limited to 0.25mm using microscope cover slips as spacers. This electrode configuration allows for up to 1mL samples within the treatment area. Figure 4.1 shows the bottom electrode of the 1mL stainless-steel chamber. Figure 4.2 depicts algae placement between the electrodes.

Figure 4.1: Bottom Stainless Steel Electrode with spacers

Figure 4.2 Side View of Stainless Steel Chamber

One advantage to this design is the ability to treat a variable (0.1 μ L to 1mL) amount of algal sample while ensuring PEF treatment of the entire sample area.

4.2 Original Capacitor Discharge Circuitry

Below is the first iteration of the circuit developed to control capacitor discharge pulses into algal sample. Resistances are in Ohms (Ω).

Figure 4.3: Repetitive Capacitor Discharge Circuit

A 5Vpp, 0V offset, 1Hz, 95% duty-cycle square wave is used to drive the circuit. When the PNP bipolar junction transistor (BJT) is on, the capacitor is charged to the DC voltage of 40V, while the load is isolated since the NPN BJT will not conduct through the collector to emitter. When the NPN is switch on, the capacitor will discharge through the load. The BJTs have continuous V_{CE} and I_C ratings of 100V and 2A, respectively.

Below is an experimental verification of the design using a 220Ω load and a 10V DC source.

Figure 4.4: Simulated Load Voltage of Repetitive Capacitor Discharge

Figure 4.4 confirms the theory of operation for the repetitive capacitor discharge circuit for a simulated load of 220Ω load and a 10V DC source. The potential difference across the simulated load was measured using two scope probes and plotted as the grey trace on the oscilloscope display.

When 100uL of *Dunaliella salina* algal solution in regular growth media is placed between the stainless-steel electrodes and used as the load, the circuit does not discharge the full capacitor potential.

Figure 4.5: 100uL Sample Load in Capacitor Discharge

The yellow, green, and grey traces are the capacitor, NPN BJT collector, and load voltage respectively. Figure 4.5 shows that the load voltage, or sample voltage, does not reach the full capacitor potential. This is assumed to be due to insufficient wave-generator current driving the NPN BJT base due to relatively large collector current. Darlington and Sziklai pairs were used to increase the base current.

4.3 Darlington and Sziklai Pair Capacitor Discharge Circuitry

To develop full charged capacitor potential across the load, a Darlington pair was used to increase the base current supplied to the NPN BJT.

Figure 4.6: Darlington Pair Repetitive Capacitor Discharge Circuit

Darlington pairs increase current gain to approximately the product of the two transistor current gains. This circuit configuration was verified experimentally with the results shown below.

Figure 4.7: Darlington Pair Capacitor Discharge Verification

Figure 4.7 shows a faster discharge time (time constant = 3ms) than Figure 4.4 for the original circuit, indicating current is discharged faster than the original circuit.

Again, 100uL of *Dunaliella salina* algal solution in regular growth media is placed between the stainless-steel electrodes and used as the load. The circuit does not discharge the full capacitor potential.

Figure 4.8: 100uL Sample Load in Darlington Pair Capacitor Discharge

Figure 4.8 shows that the Darlington pair configuration allows for more potential (2V more) to develop across the load than the original circuit. However, the voltage developed across the load is still only 5V, half the 10V DC supply. To further increase input base current, a Sziklai pair was coupled with the Darlington pair shown in Figure 4.9.

Figure 4.9: Sziklai-Darlington Pair Repetitive Capacitor Discharge Circuit

A Sziklai pair has similar current capability to the Darlington pair, but with a lower turn on voltage. Again, this circuit was used to increase current through the load. This circuit's operation was verified using a 220Ω load with the results shown Figure 4.10.

Figure 4.10: Sziklai-Darlington Pair Capacitor Discharge Circuit Verification

The discharge time of the Sziklai-Darlington pair was over twice as long (time constant = 6ms) as that of the Darlington pair, and a constant voltage across the emitter and collector of the output PNP is observed, causing a slight voltage drop across the sample load.

Figure 4.11 shows that the Sziklai-Darlington pair, as indicated by the verification test above, was not able to yield more voltage drop across the load than the Darlington pair for a 100uL *Duneliella* sample.

Figure 4.11: 100uL Sample Load in Sziklai-Darlington Pair Capacitor Discharge

4.4 Algae Sample Conductance and Parallel Plate Capacitance

The failure of the three discharge circuits may be due to multiple load aspects: variability of algae solution conductance with applied voltage and parallel plate capacitance.

In several attempts to quantify the conductivity of the algae solution, it was found that conductivity of the sample increases with voltage. Since the sample tended to heat and boil with sustained DC voltage, simple methods of reliably quantifying conductivity for sustained DC was not found.

Secondly, each circuit was tested with a $33\mu\text{F}$ capacitor in parallel with a 220Ω resistor to attempt to simulate the effective capacitance of the parallel plates used in the PEF chamber. The results below show that the parallel capacitance effectively reduces the voltage across the

sample. This is due to the near zero initial capacitor resistance ; therefore, this is most likely the cause for the initial voltage drop observed in previous discharge experiments.

Figure 4.12: Original Circuit with Parallel $33\mu\text{F}$ Capacitor and 220Ω Resistor Load

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Figure 4.13: Darlington Pair with Parallel $33\mu\text{F}$ Capacitor and 220Ω Resistor Load

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Figure 4.14: Sziklai-Darlington Pair with Parallel $33\mu\text{F}$ Capacitor and 220Ω Resistor Load

Experiment Conclusions

The results imply that a PEF chamber with limited parallel capacitance is necessary. However, this is difficult to achieve while maintaining close 0.25mm electrode spacing for 100 μ L sample volumes. Electrical characteristics - permittivity and conductivity - of the *Dunaliella* media may be too large, inducing large parallel capacitance and exceeding NPN BJT drive current requirements from the wave-generator.

Chapter 5: Lysis Thresholds for *Chlorella vulgaris*

A 0.25mm spaced stainless steel electrode chamber (Figure 4.2) was used to repetitively discharge capacitor banks at a frequency of 3Hz into centrifugally concentrated *Chlorella vulgaris* in regular growth solution. Each sample was treated for 20s, corresponding to approximately 60 applied pulses. The PEF chamber sample is 10uL. Table 5.1 shows signal characteristics for pulse patterns applied to three different samples. Previous tests were conducted (data not shown) at lower voltages (40V-50V), however, not effective without sufficiently long pulse durations, which caused heating.

Test	Capacitor Voltage	Max Electric Field	Equivalent Series Capacitance	Applied Energy
1	80V	3.2kV/cm	8.25uF	1.58 J
2	100V	4kV/cm	8.25uF	2.475J
3	100V	4kV/cm	2uF	0.6 J

Table 5.1: Signal Characteristics for *Chlorella* Treatment

Test one was did not sufficiently lyse cells. Figure 5.1 shows that a majority of cell walls are still intact.

Figure 5.1: Chlorella after 60 3.2kV/cm pulses at 3Hz

Test two was the most successful in lysing *Chlorella*; cells in Figure 5.2 have the appearance of a fractured cell wall.

Figure 5.2: *Chlorella* after 60 4kV/cm pulses with 8.25uF Capacitor at 3Hz

Figure 5.3: *Lysed Cell From Test Two*

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Figure 5.4: Discharge Waveform for Test 2

Test 3 was successful in lysing cells. However, it is not as pronounced as in Test 2.

Figure 5.5: *Chlorella* after 60 4kV/cm pulses with 2uF Capacitor at 3Hz

Experiment Conclusions

It was shown that a single 4kV/cm electric field pulse was sufficient to lyse *Chlorella vulgaris* cells. However, cell membrane deterioration is not observed as in previous *Dunaliella* tests. This is most likely due to *Chlorella* cellulose walls. Additional PEF application may be necessary to sufficiently release lipids through the cell membrane and cellulose wall.

Ch. 6: Results Discussion

Table 6.1 specifies capacitor discharge parameters and minimum electric field magnitudes found to lyse *Dunaliella salina* and *Chlorella vulgaris*.

Species	Minimum Electric Field Intensity	Discharge Time Constant (τ)	Pulse Number	Single Pulse Energy
<i>Dunaliella salina</i>	1.6kV/cm	100ms	5	52.6 mJ
<i>Chlorella vulgaris</i>	4kV/cm	160us	60 (min. number not optimized)	41.25 mJ

Table 6.1: Minimum Signal Characteristics Lysis Summary

It may be possible to optimize energy expenditure by increasing voltage and decreasing pulse duration and number, as observed with a single 8kV/cm 8.8uJ pulse applied to *Dunaliella*. Additionally, *Chlorella*'s cellulose wall may be difficult to significantly disrupt with PEF at the magnitudes listed. It is clear that the results can be further optimized to minimize heat applied to the sample, total energy expenditure, and effectiveness.

Utilizing eq. 1.4 for transmembrane potential

$$V_m = -1.5E_o b \cos \vartheta \quad (1.4)$$

Figure 6.1: Cell Model [2]

and considering the cell membrane potential normal to the induced uniform electric field,
 $\theta = 0^\circ$ or 180° .

$$|V_{m\perp}| = 1.5E_o b \quad (6.1)$$

Table 6.1 specifies membrane potential normal with respect to radius and minimum field magnitude necessary to lyse cells.

Species	Minimum Electric Field Intensity	Cell Radius	$ V_{m\perp} $
<i>Dunaliella salina</i>	1.6kV/cm	4.5 μm – 5.5 μm	1.05V – 1.32V
<i>Chlorella vulgaris</i>	4kV/cm	1 μm - 5 μm	0.6V – 3V

Table 6.2: Membrane Potential for Lysis

Assuming the median radius is the average, a transmembrane potential of 1.8V is required to lyse *Chlorella* and 1.2V to lyse *Dunaliella*. This corresponds to the critical voltage $V_m \cong 1.5\text{V}$ predicted by Zimmeraman [3]. The required *Chlorella's* membrane potential may be greater because of the cell's cellulose layers. From Table 6.2, it appears that Eq. 1.4 may be useful in predicting minimum required electric field magnitudes for cell lysis based on algal radii.

Conclusion and Future Work

The thesis has successfully identified electric field signal parameters including magnitude, duration, and number of pulses required to lyse two algal species. It also serves as a proof of concept for the use of PEFs to lyse algal cells. The PEF technique provides the basis for designing larger continuous flow chambers when considering maximum power supply voltage and electrode spacing, while pulse width effectiveness provides estimates on maximum flow rate.

Further work on lysing algal cells can be focused on increasing electric field magnitude and using square wave pulses can optimize pulse energy.

Additional refinements are needed for commercially viable algae based biofuel; however, this work can serve as a basis for implementing larger harvesting and processing systems.

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APPENDIX A: Transmembrane Potential Derivation

Figure A.1 Pohl [2] Two Layered Cell Model

Define complex permittivity

$$\zeta_k = \varepsilon_k - j \frac{\sigma_k}{\omega} \quad (\text{A.1})$$

Considering the boundary condition between two layers

$$\hat{n} \cdot (\mathbf{D}_1 - \mathbf{D}_2) = \rho_s$$

Since the media is considered to be non-perfect conductors ($\sigma \ll \infty$), it is assumed $\rho_s \rightarrow 0$

$$\hat{n} \cdot \mathbf{D}_1 = \hat{n} \cdot \mathbf{D}_2$$

Given constitutive relation

$$\begin{aligned} \mathbf{D} &= \zeta \mathbf{E} \\ \zeta_1 (\hat{n} \cdot \mathbf{E}_1) &= \zeta_2 (\hat{n} \cdot \mathbf{E}_2) \end{aligned}$$

Using the scalar potential expression for electric fields

$$\begin{aligned} \mathbf{E} &= -\nabla \Phi & (\text{A.2}) \\ \zeta_1 (\hat{n} \cdot \nabla \Phi_1) &= \zeta_2 (\hat{n} \cdot \nabla \Phi_2) & (\text{A.3}) \end{aligned}$$

The boundary normal is in the radial direction

$$\hat{n} = \hat{r}$$

$$\zeta(\hat{\mathbf{r}} \cdot \nabla \Phi) = \zeta \frac{\partial \Phi}{\partial r}$$

From Eq. (A.3)

$$\zeta_1 \frac{\partial \Phi_1}{\partial r} = \zeta_2 \frac{\partial \Phi_2}{\partial r} \quad (\text{A.4})$$

Considering imaginary terms of ζ from Eq. (A.4)

$$\sigma_1 \frac{\partial \Phi_1}{\partial r} = \sigma_2 \frac{\partial \Phi_2}{\partial r} \quad (\text{A.5})$$

Now, considering the boundary condition for tangential electric fields

$$\hat{\mathbf{n}} \times (\mathbf{E}_1 - \mathbf{E}_2) = 0$$

Substituting scalar potential

$$\hat{\mathbf{n}} \times (\nabla \Phi_1 - \nabla \Phi_2) = 0$$

Therefore

$$\Phi_1 = \Phi_2 \quad (\text{A.6})$$

The radial component of the electric field is given by

$$E_r = E_o \cos \theta$$

Considering an expression for the field without cells, from Eq. (A.2)

$$\mathbf{E} = -\frac{\partial \Phi}{\partial r} \hat{\mathbf{r}} - \frac{1}{r} \frac{\partial \Phi}{\partial \theta} \hat{\boldsymbol{\theta}}$$

Assuming no ϕ field variations

$$\begin{aligned} E_r &= -\frac{\partial \Phi}{\partial r} \\ \frac{\partial \Phi}{\partial r} &= -E_o \cos \theta \end{aligned} \quad (\text{A.7})$$

Integrating Eq. (A.7) with respect to r yields

$$\Phi = -E_o r \cos \theta \quad (\text{A.8})$$

for the scalar potential without cells.

When a cell is placed within the field, fields outside and inside the cell retain symmetry, remaining independent of ϕ . The potential of a sphere in the presence of an electric field is expressed in terms of Legendre functions for each region [4]

$$\Phi_i = \sum_{n=0}^{\infty} \left(A_{in} r^n + \frac{B_{in}}{r^{(n+1)}} \right) P_n(\cos \theta) \quad (\text{A.9})$$

where $P_n(\cos \theta)$ are Legendre functions and A_{in} and B_{in} are coefficients determined by the boundary conditions of Eqs. (A.5) and (A.6).

Considering boundary condition (A.5)

$$\sigma_i \frac{\partial \Phi_i}{\partial r} = \sigma_i \sum_{n=0}^{\infty} \left(n A_{in} r^{n-1} - (n+1) \frac{B_{in}}{r^{(n+2)}} \right) P_n(\cos \theta)$$

For a uniform field as $r \gg a_3$ (i.e. for fields past the cell layer, see Figure A.1), $\Phi = -E_o r \cos \vartheta$, therefore $n = 1$ and $P_1(\cos \vartheta) = \cos \vartheta$.

$$\Phi_i = \left(A_{i1} r^1 + \frac{B_{i1}}{r^2} \right) \cos \theta \quad (\text{A.10})$$

$$\sigma_i \frac{\partial \Phi_i}{\partial r} = \sigma_i \left(A_{i1} - 2 \frac{B_{i1}}{r^3} \right) \cos \theta \quad (\text{A.11})$$

Since the field is uniform for $r \gg a_3$ and

$$\Phi = -E_o r \cos \theta$$

for the original potential, $A_{41} = -E_o$ ($r \gg a_3$). Therefore

$$\Phi_4 = \left(-E_o r + \frac{B_{41}}{r^2} \right) \cos \theta$$

using (A.5) boundary condition for $\sigma_4 \frac{\partial \Phi_4}{\partial r} = \sigma_3 \frac{\partial \Phi_3}{\partial r}$ for $r = a_3$

$$\sigma_4 \left(-E_o - 2 \frac{B_{41}}{a_3^3} \right) \cos \theta = \sigma_3 \left(A_{31} - 2 \frac{B_{31}}{a_3^3} \right) \cos \theta$$

Assuming that $\sigma_3 \rightarrow 0$ for a non-conducting cell membrane, the above equation simplifies to

$$B_{41} = -\frac{E_o a_3^3}{2}$$

Therefore, the scalar potential for $r \geq a_3$ is

$$\Phi = -E_o \left(r^1 + \frac{a_3^3}{2r^2} \right) \cos \theta \quad (\text{A.12})$$

For the transmembrane potential, where $r = a_3$

$$\begin{aligned} \Phi_4 &= -E_o \left(a_3^1 + \frac{a_3^3}{2a_3^2} \right) \cos \theta \\ \Phi_4 &= -1.5 E_o a_3 \cos \theta \end{aligned} \quad (\text{A.13})$$

Notice that Eq. (A.13) is the same expression given by Zimmerman [1] for transmembrane potential in Eq. (1.4). This shows that multiple layered cells, assuming the $\sigma_{k-1} \ll \sigma_k$ (where σ_k is the medium and σ_{k-1} is the cell's outer layer), have the same membrane potential as cells with single layers. The theta term shows that membrane potential is greatest at the cell membrane boundary normal to the electric field, while minimal at the boundary parallel to the electric field.