Pulsed Electric Field System Development for Algae Biofuel Extraction

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Abstract

The search for sustainable alternatives to fossil fuels proves necessary due to the effects fossil fuels have on Earth's environment. These alternatives must have a minimum ecological footprint and cause no long-term harm to the environment. An environmentally friendly fuel is necessary to usher in a generation of renewable green energy.

The production of algae refined biofuels becomes a possible solution to this growing issue. Algae absorb CO_2 from the atmosphere during their reproduction and growth cycles, yielding a positive environmental impact. As the biofuel is refined and becomes suitable for use, the combustion of the finished fuel generates an equal amount of CO_2. Thus, yielding a net zero environmental impact.

Algae biofuel is fabricated from the intracellular materials of algae cells. This report describes the application of pulsed electric fields (PEF) to algae for intracellular extraction. PEF treatments rupture the algae cell membrane allowing a centrifuge to extract lipid-rich contents from the interior cell. **Fig. 1** shows the algae cell's biochemistry.

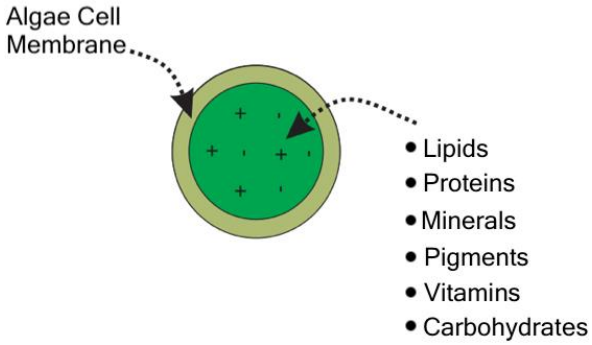

FIGURE 1: ALGAE CELL INTERIOR

Introduction

Cultivation of Chlorella Vulgaris

The research team harvests, conserves, and maintains Chlorella Vulgaris cultures in glass flasks placed on a digital shaker below florescent lighting, as shown in **Fig. 2**. The algae cultures are harvested from an original culture obtained from a vendor. The culture is then split to create additional cultures. When the culture is split, proper sanitation and sterilization lab procedures prevent the introduction of other organisms and pollutants. The split culture and media (Bold's Basal Medium or BBM) are added to a newly sterilized flask. The flasks are sealed with aluminum foil and cotton, placed under fluorescent lighting, and given enough BBM to reproduce and grow freely. Multiple cultures with different maturity levels (culture age) are maintained to guarantee constant algae samples for testing. The small 25mL flasks are used because the PEF treatments performed in this report utilize between 0.3 - 0.5mL from the cultures. New C. Vulgaris cultures contain 9mL of algae media (BBM) and 1mL of a previous healthy culture (a culture without contaminants and low algae density).

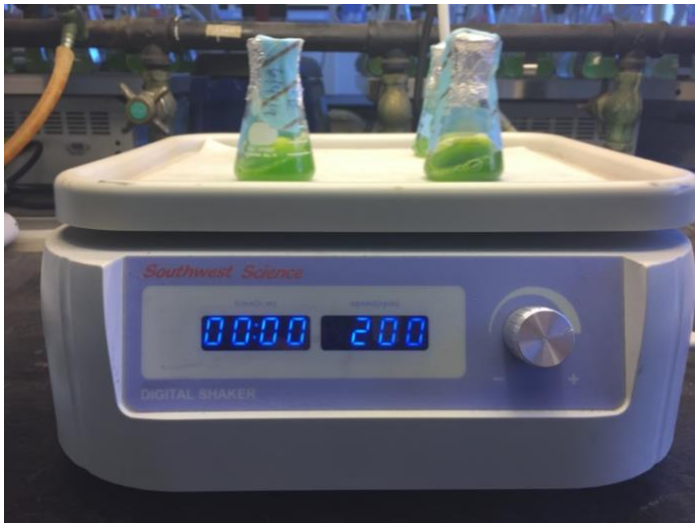

FIGURE 2: CHLORELLA VULGARIS ALGAE CULTIVATION SETUP

Lysis Via Lyse-It kit

The cultivated cells of an algae sample before lysis are pictured in **Fig. 3.** The Biology team has achieved lysis using a ***Lyse-It™*** kit, **Fig 4a**. [1] The results are compared to our lysis attempts with the designed Electroporator. The ***Lyse-It™*** kit focuses microwave energy directly into a C. Vulgaris sample by utilizing single use slides. A foam gasket is attached to a ***Lyse-It™*** slide with gold foil to focus the microwave energy directly into the sample. 1mL of algae is placed into the gasket and a plastic coverslip added. The sample is placed in the middle of the microwave oven and microwaved for 30 seconds with the power level set to 2 (20%). The C. Vulgaris cells after utilizing ***Lyse-It™*** kit are represented in **Fig. 4b**.

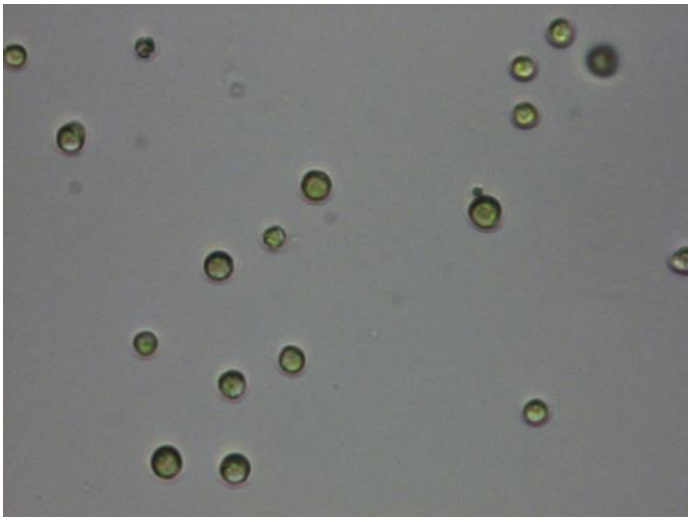

FIGURE 3: CHLORELLA VULGARIS BEFORE LYSIS

FIGURE 4A: LYSE-IT KIT

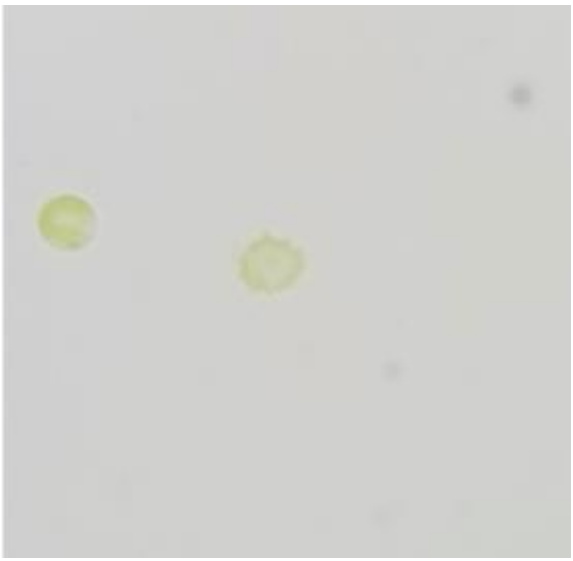

FIGURE 4B: LYSIS VIA LYSE-IT KIT

PEF Treatment Chamber Design

Without a ***Lyse-It™*** kit, the electric field necessary to rupture the algae cell wall is created by an electroporator device or pulse generator. Pulsed electric field (PEF) treatments are accomplished by placing the algae in a constructed electroporator chamber. **Fig. 5** shows the chamber we have constructed with Indium Tin Oxide (ITO) coated glass slides and Kapton tape.

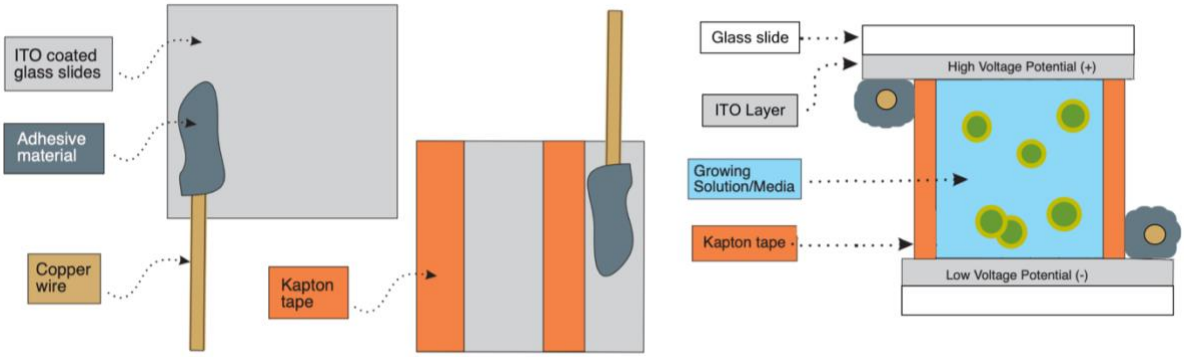

FIGURE 5: TOP VIEW (LEFT) AND SIDE VIEW (RIGHT) OF THE CONSTRUCTED CHAMBER

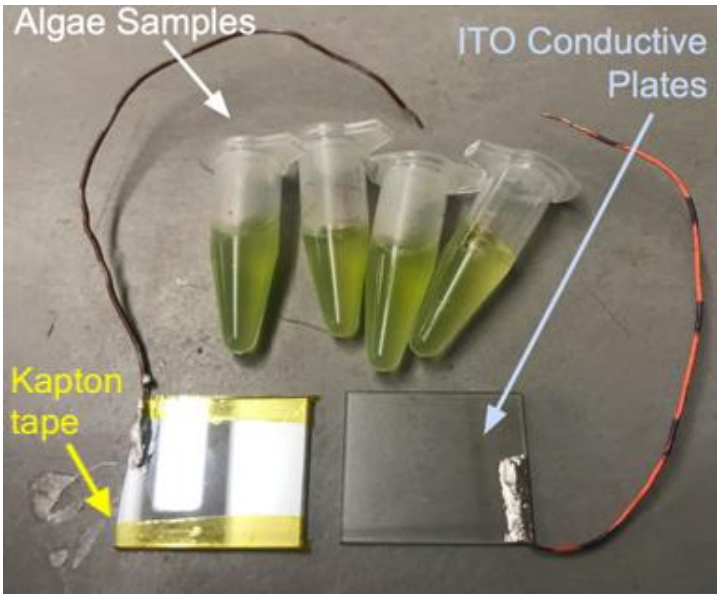

FIGURE 6: ITO PEF TREATMENT CHAMBER

The PEF treatment chamber, **Fig. 6**, is assembled by clamping the two ITO conductive sides together. The ITO coating allows the glass slides to conduct electricity. The Kapton tape prevents the two ITO coated slides from touching. If the ITO coated slides touch, this would cause a short circuit that would destroy the chamber. The slides are connected to the electroporator with 24 AWG gauge wire and liquid solder. The clear slides allow for the observation of the algae through a microscope. To create the PEF, a voltage potential is distributed across the conductive chamber slides. This process is illustrated in **Fig. 7**.

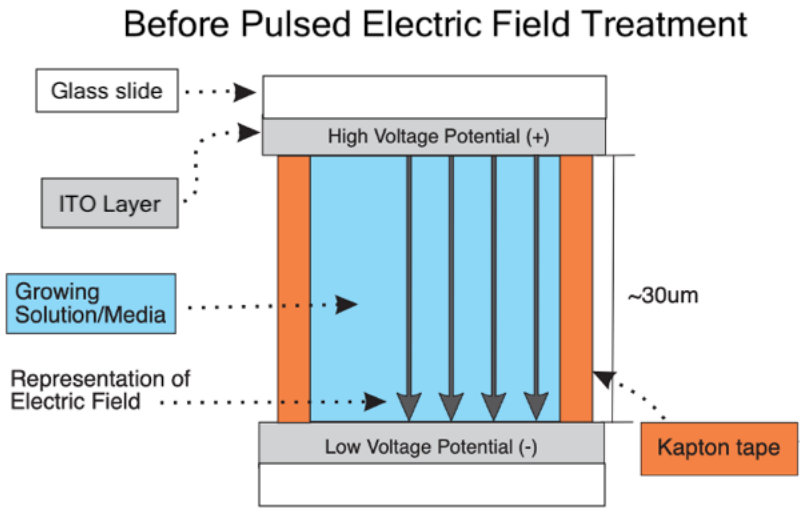

FIGURE 7: ELECTROPORATOR'S PEF TREATMENT CHAMBER DIAGRAM

The electric field in **Fig. 7** is proportional to the voltage potential (1) between the slides and their calculated distance (2).

$$V_{slides}\ (Volts) = V_{+} - V_{-} \quad (1)$$

$$E\ (KV/cm) = V_{slides}\ (V) / d\ (\mu m) \quad (2)$$

Subjecting the slides to a voltage potential from 0V up to 140V yields PEF intensities up to ~46.67(KV/cm), calculated using Equation (2). This method presents limitations that include: costly glass chamber fabrication (ITO glass slides cost $15 a piece) and possible short circuiting (ITO damage).

In theory, the creation of pores in the algae's membrane occurs after the electric field value calculated above is applied. [2] [3] However, the algae are capable of healing their cellular membrane by closing their pores. Algae biofuel production requires that the electric field applied to the algae surpass the critical electric field necessary to prevent the algae healing process. **Fig. 8** describes the three possible outcomes when algae cells are subjected to an electric field.

Fig. 8 demonstrates that algae cell lysing requires an electric field level greater than the critical threshold. Researchers hope to find the pulsed electric field characteristics necessary to cause irreversible pore reformation, making lipid extraction and the fuel refinement process of algae biofuel sustainable. The pulsed electric field characteristics include:

- Pulse Shape
- Period
- Repetition rate
- Pulsewidth
- Amplitude

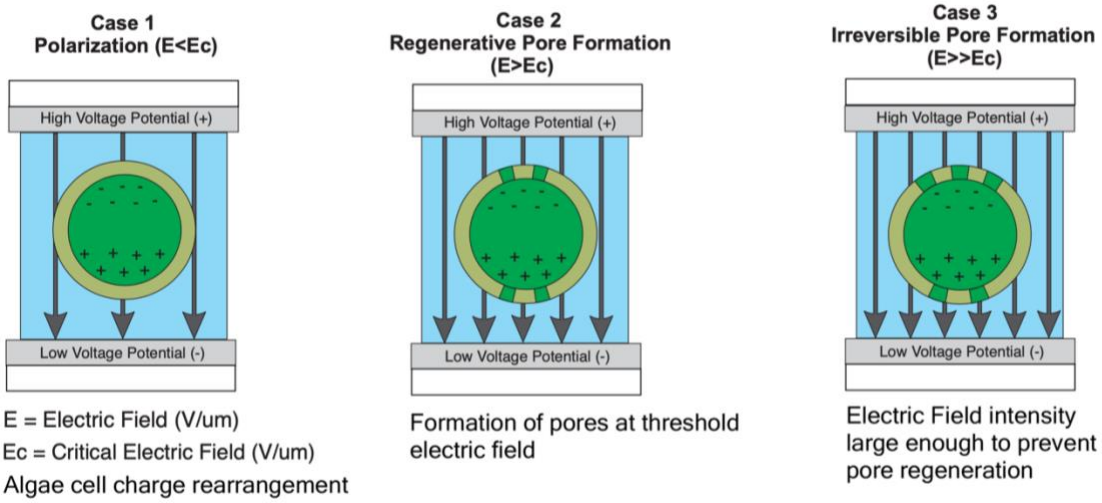

FIGURE 8: POTENTIAL PEF TREATMENT CASES [4].

Finding the exact pulse parameters, materials used, and electroporation methods that assert the algae to irreversible pore formation are crucial for understanding the algae and PEF characteristics that produce lysis [4]. Irreversible pore formation is depicted in Case 3 of **Fig. 8**. This state of cell electroporation is critical because it allows centrifuging of the lysed cells and extraction of intracellular materials. The process for obtaining large quantities of intracellular materials utilizing PEFs remains an obstacle.

Algae Impedance Model

Previous research conducted by Cal Poly's Algae Biofuel team concluded that the algae impedance can be represented by a combination of capacitance and resistance illustrated in **Fig. 9**. This model is not completely accurate because the algae impedance can vary based on algae concentration, volume, and sample size. The impedance model was derived to help with the simulation and construction of an electroporator device.

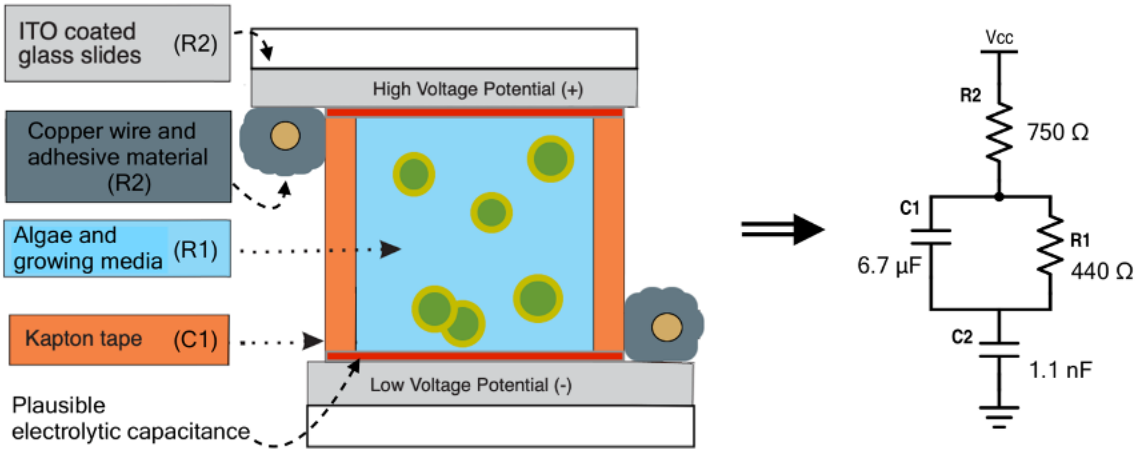

FIGURE 9: THEORIZED IMPEDANCE MODEL OF THE ITO CHAMBER WITH ALGAE

Equivalent Circuit Design of Electroporator

The Electroporator (Pulse Generator) device applies PEF treatments to an algae sample using the charging and discharging capabilities of a capacitor. The Level 0 Block Diagram of the system is in **Fig. 10**.

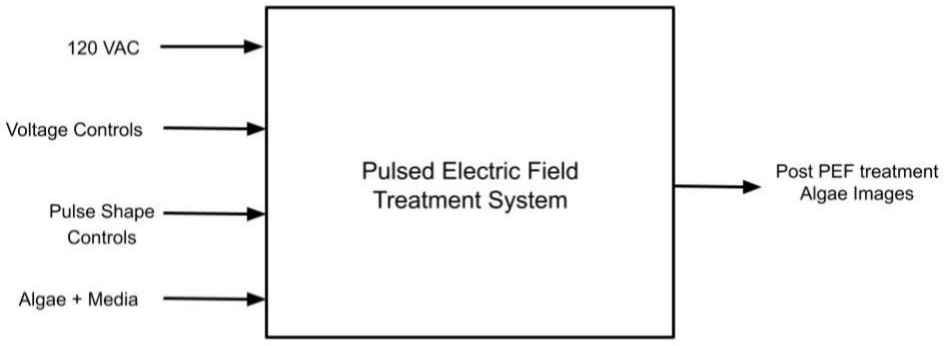

FIGURE 10: LEVEL 0 BLOCK DIAGRAM, ALGAE CELL LYSIS ELECTROPORATION SYSTEM

Fig. 11 describes the level 1 block diagram of the Electroporator circuit. Creating two Pulse Generator's that utilize a mechanical relay and a solid-state relay. Equation (3) shows the RC time constant where the potentiometer is controlled by the user to obtain a specific tau (τ) value.

$$\tau = R_{potentiometer} C_{storage} \quad (3)$$

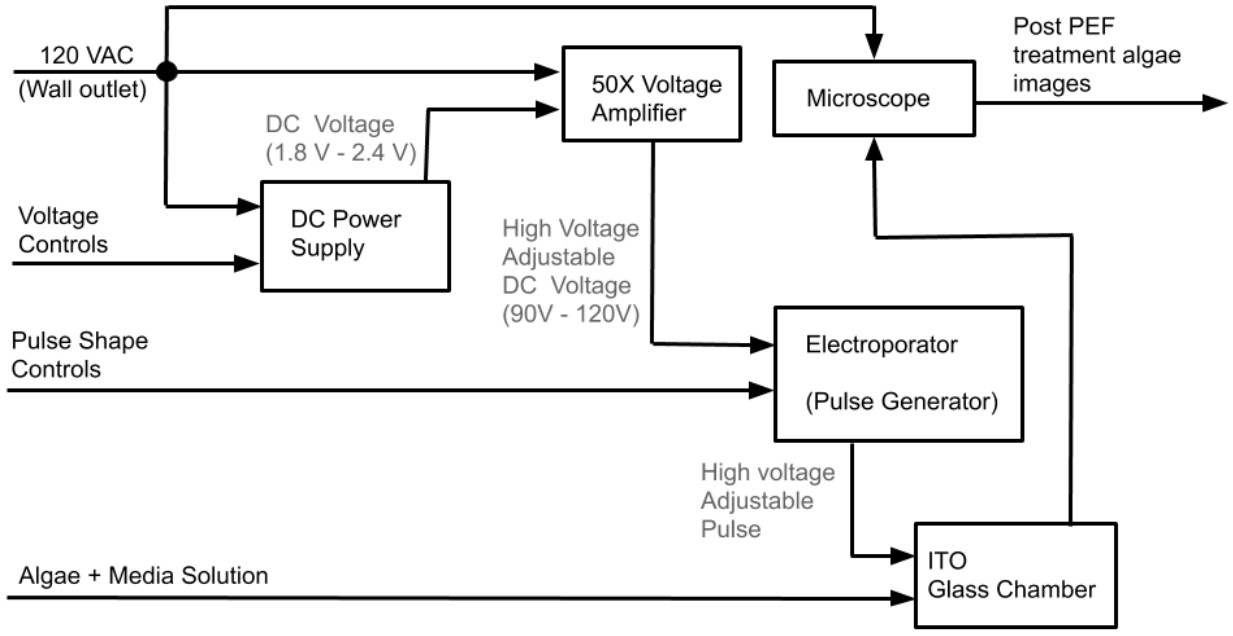

FIGURE 11: LEVEL 1 BLOCK DIAGRAM, ALGAE CELL LYSIS ELECTROPORATION SYSTEM

The equivalent circuit of our Mechanical Relay Electroporator device is shown in **Fig. 12**. The circuit shows a Power Source of 90V – 120V that is supplied to a 1kΩ Charging Resistor. When the relay is in the normally closed (NC) the supplied voltage goes through the Charging Resistor and into the Storage Capacitor to charge it. When the relay is switched to NO, the voltage in the Storage Capacitor is discharged across the constructed Algae Chamber in parallel with a Potentiometer. The Storage Capacitor is a fixed value in the circuit while the potentiometer is controlled by the user. The Mechanical Relay Electroporator allows more precision of the pulse widths to be in the 1us-10us range, but the relay's switching time is slow since it takes 0.5s to switch. This makes it challenging to inject a train of pulses to lyse the algae. The minimum pulse repetition time with the mechanical relay is about 0.5 seconds.

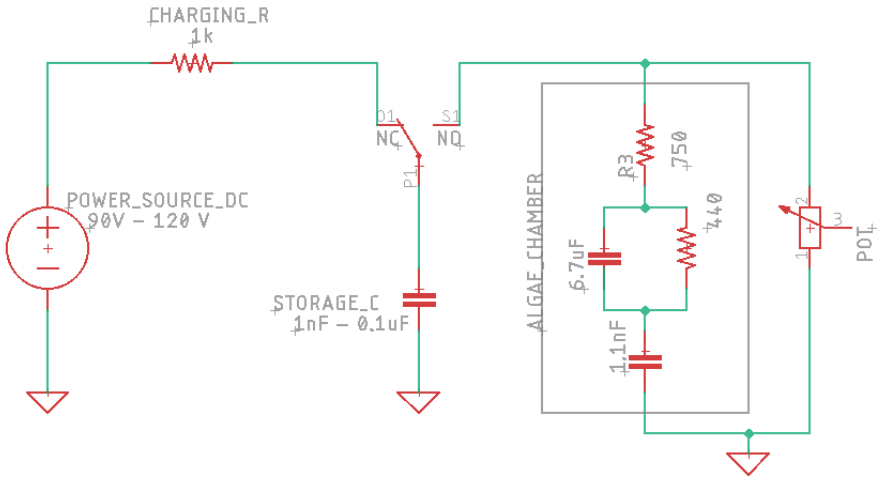

FIGURE 12: ELECTROPORATOR MECHANICAL RELAY EQUIVALENT CIRCUIT

The second pulse generator is constructed with solid state relays (SSRs) controlled by an Arduino. The Arduino Code commands the pulse widths and allows a train of pulses to be injected into the algae. The SSR circuit leads to more issues such as a drop in voltage of 80% seen across the algae. This drop in voltage is due to the opto-TRIAC in the SSR and an inaccurate impedance model of the ITO + algae. The equivalent circuit of the Solid State Relay Electroporator is shown in **Fig. 13**.

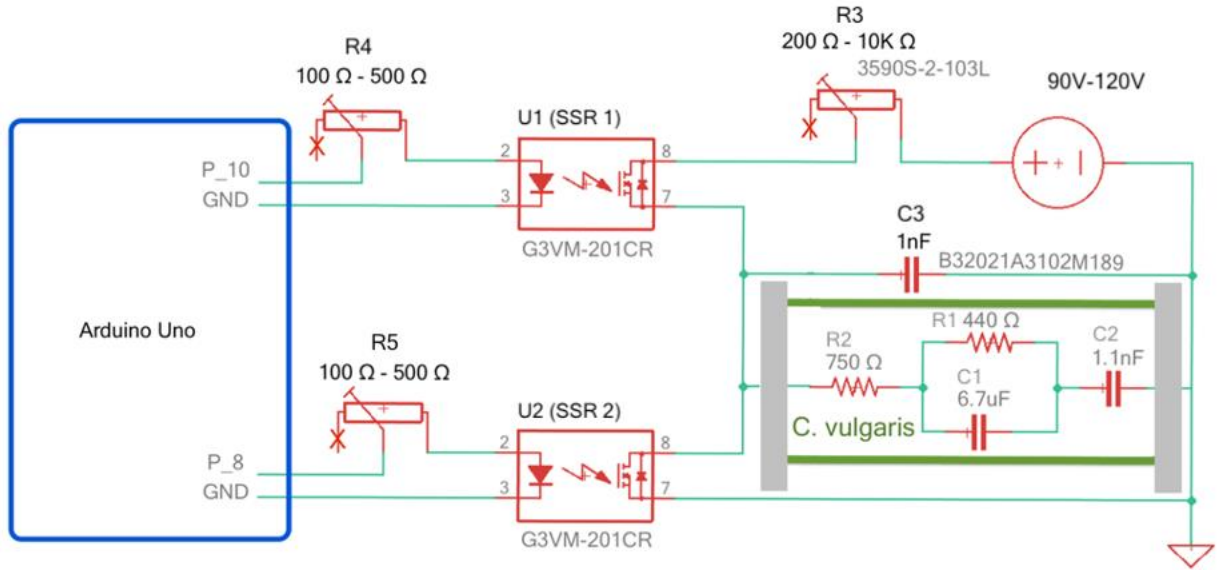

FIGURE 13: SOLID STATE RELAY ELECTROPORATOR CIRCUIT DIAGRAM

TABLE 1
SYSTEM REQUIREMENTS AND SPECIFICATIONS FOR ALGAE ELECTROPORATOR DEVICE

Marketing Requirements	Engineering Specifications	Justification
1, 3	Deliver pulses with microsecond widths, 1us-10us, voltages 90V-140V and fields strengths of ~46.67[$\frac{KV}{cm}$]	Previous research to achieve algae lysis based on past studies [4] [5]
5	Source, 120V AC	Available Power Sources
4	User controlled pulse characteristics: length, duration, voltage peak, frequency, and strength	Allows optimum combination for effective lysis
1, 3	Electroporator emits a pulse train with the specified user inputs.	Allows user to discharge more than a single pulse into the algae sample

Marketing Requirements

1. Adjustable output pulse characteristics
2. User-friendly interface
3. Accommodates different algae densities
4. Customer Safety
5. Utilizes commercial power source

The mechanical relay circuit consists of a power source in series with a charging resistor with a value of 1 kΩ connected to the normally closed (NC) arm of the relay. When triggered, the relay switches to the normally open (NO) position and distributes the storage capacitors charge across the algae chamber and electroporator. The schematic of the circuit appears in **Fig. 14.**

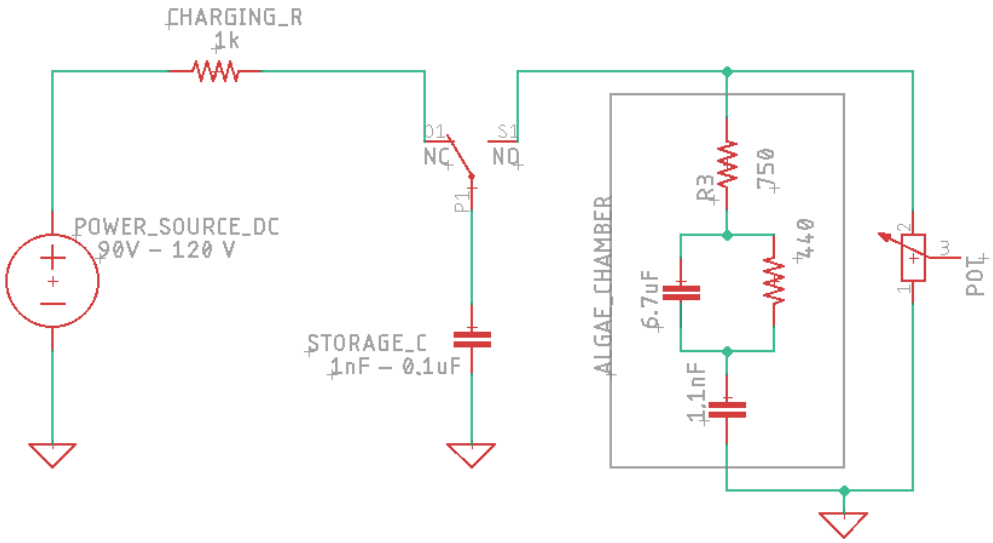

FIGURE 14: ALGAE ELECTROPORATOR MECHANICAL RELAY CONFIGURATION CIRCUIT DIAGRAM

The relay in **Fig. 14** is controlled by a NPN Relay Switch Circuit to help eliminate the bouncing from the relay's mechanical arm. Bouncing occurs when the relay has first been switched but not yet settled, which can cause the circuitry to react as if the relay had been toggled multiple times. The NPN Relay Switch Circuit configuration is shown in **Fig. 15**.

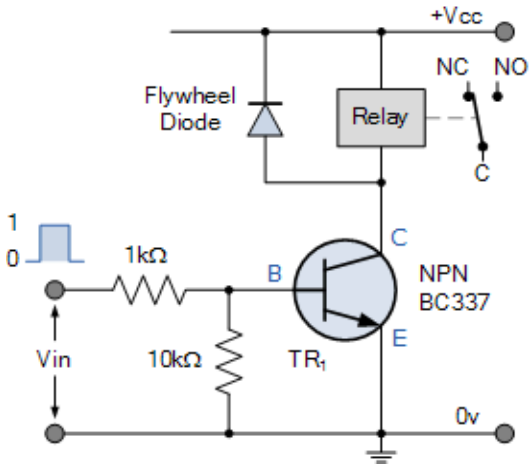

FIGURE 15: NPN RELAY SWITCH CIRCUIT

The initial design of the circuit utilized a 1nF storage capacitor and 1 kΩ-3 kΩ potentiometer value to achieve a time constant in the microsecond range. After testing the circuit, the results yielded a time constant in the millisecond range. Combining the measured time constant results with the potentiometer value, calculated algae resistance is 125.8Ω - 128.5Ω using equation (4) and (5).

$$\tau = R_{total} C_{storage} \quad (4)$$

$$\frac{1}{R_{total}} = \frac{1}{R_{algae}} + \frac{1}{R_{potentiometer}} \quad (5)$$

A table of resistor values was calculated utilizing equation (4) and (5). The resistor values were calculated using an estimated value of 126 Ω for the algae sample, a time constant in the range of 1us-10us, and capacitor values between 1uF-1nF. These resistances represent the value the potentiometer should be set to when taking the resistance of the parallel algae sample into consideration.

TABLE 2
POTENTIOMETER RESISTANCES FOR SPECIFIED CAPACITOR AND TIME CONSTANT VALUES

	1us	2us	3us	4us	5us	6us	7us	8us	9us	10us
1uF	1	2	3	4	5	6	7	8.5	9.7	10.0
0.1uF	10.9	23.8	39.48	58.6	82.9	114.6	157.5	129.1	315	485.6
0.047uF	25.6	64.25	129.4	262.2	683.3	-9692	-818.2	-485	-368.4	-309
10nF	485	-340.5	-217.2	-183.9	-168.5	-159.5	-153.7	-149.6	-146.5	-144.2
1nF	-144	-134.5	-131.5	-130	-129.3	-128.7	-128.3	-128	-127.8	-127.6

LEGEND

τ Values	Capacitances Values	Not Possible Ω	Possible Ω

Reviewing the values in Table 2 shows a 0.1uF storage capacitor will facilitate the largest testing range. It was also concluded that the incorrect time constant was observed across the algae for the 1nF in previous testing. A negative potentiometer resistance was needed in order to achieve a time constant in the 1us-10us range, which is not possible

Mechanical Relay Electroporator Simulation

The mechanical relay electroporator is simulated using LTSpice. The constructed circuit is represented in **Fig. 16**. LTSpice does not have a relay component so two switches are used to mimic the behavior of a relay. The leftmost switch functions as the normally closed pole (NC) of the relay and the right switch functions as the normally open pole (NO). The algae sample is modeled by a 126 Ω resistor (as mentioned above), which is the value used to calculate the resistance values in Table 2. The discharging resistor in **Fig. 16** is varied according to the calculated resistance values in Table 2 for the 0.1uF capacitor. **Fig. 17-19** display the results for time constant values of 1us, 5us, and 10us as measured across the algae sample. All the results for 1us-10us can be seen in Appendix A.

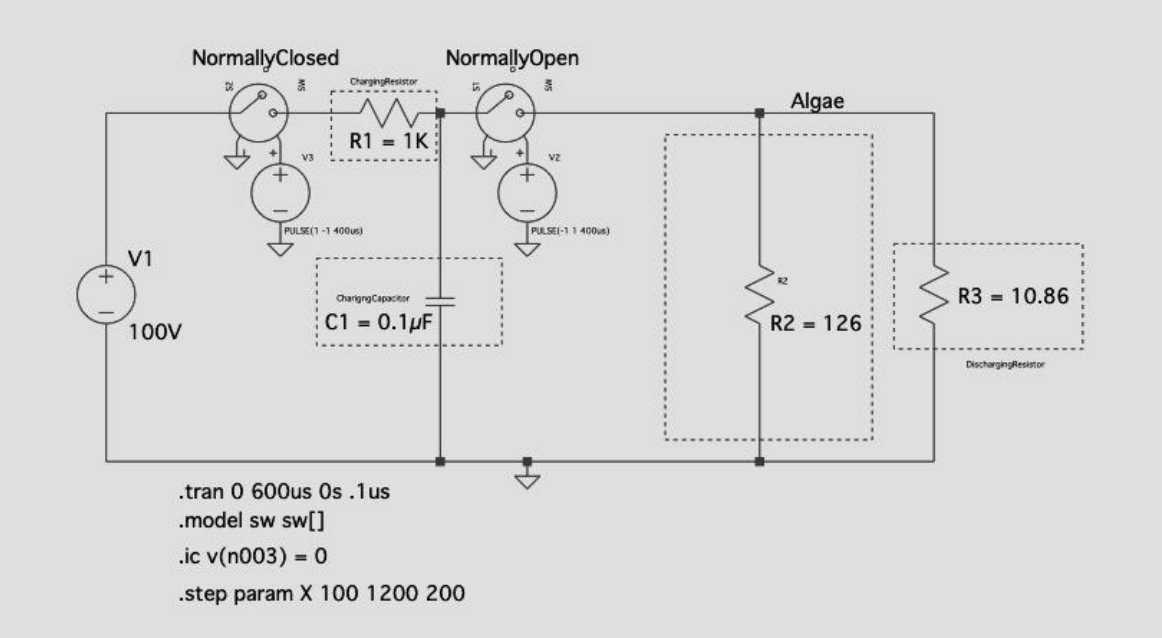

FIGURE 16: LTSPICE MECHANICAL RELAY ELECTROPORATOR CIRCUIT

The exponential decay equation states that the time constant of the waveform can be measured as the value between the peak voltage and 37% of the peak voltage. [6] The cursers are used to measure between the two voltages to obtain the time constant. The calculated time constant is boxed in green for each of the figures.

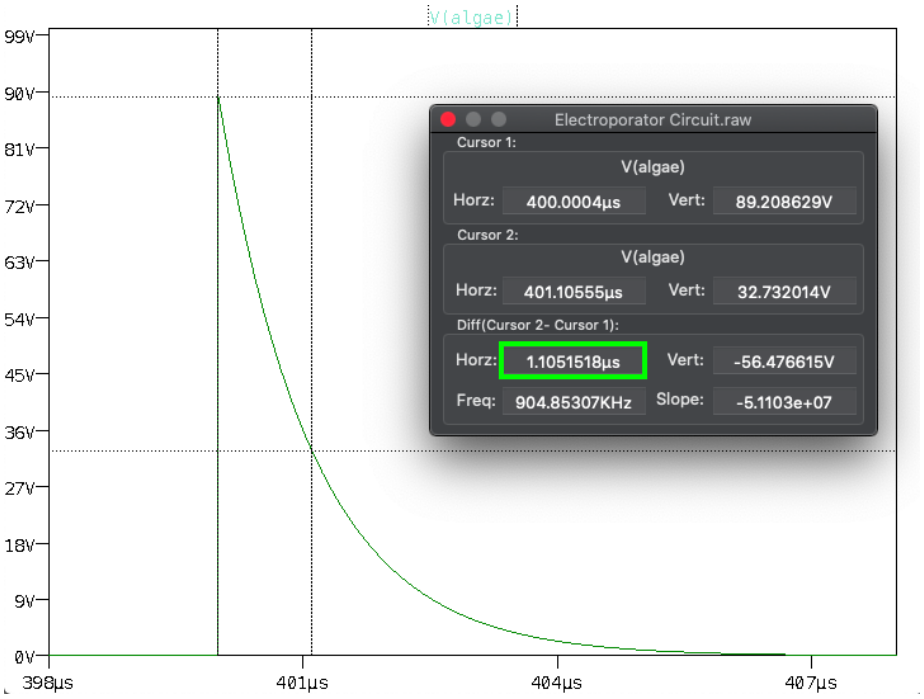

FIGURE 17: 1US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

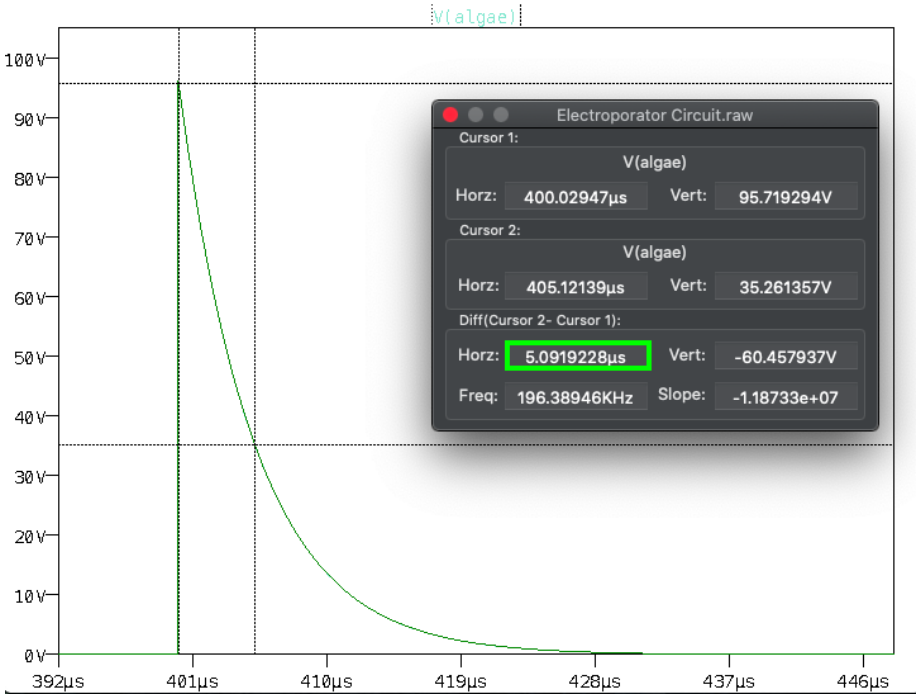

FIGURE 18: 5US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

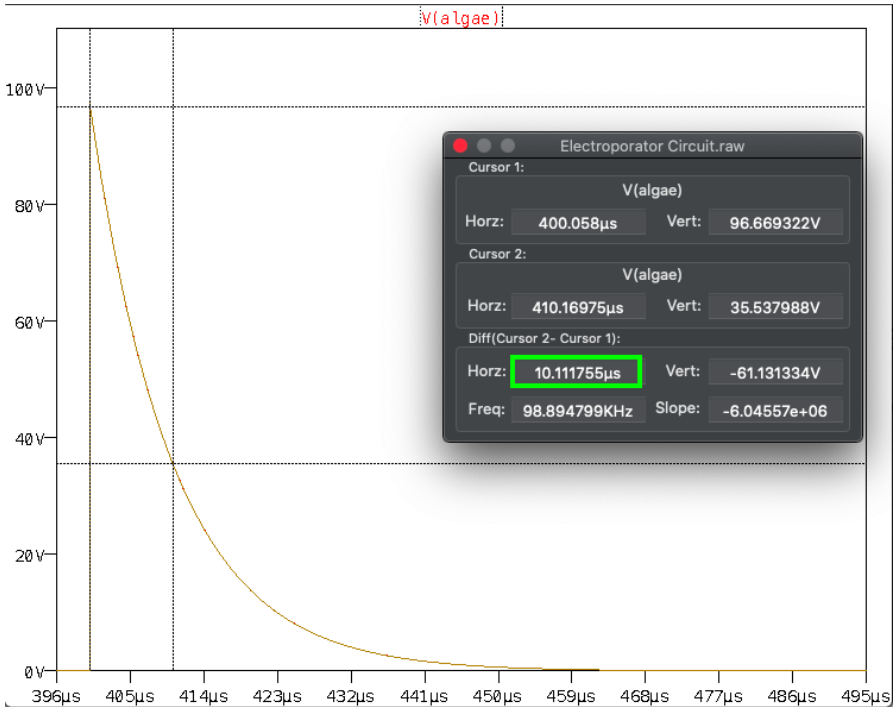

FIGURE 19: 10US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

Mechanical Relay Electroporator Data

Initial testing of the mechanical relay electroporator circuit was conducted at a voltage of 15V to ensure proper pulse shape, as shown in **Fig 17-19,** was produced. Measurements were taken to achieve the 1us-10us time constants by adjusting the potentiometer to match the resistor values in Table 2 as close as possible. An oscilloscope is used to measure the voltage across the potentiometer both with and without an algae sample present.

Fig. 20 shows the voltage measured across the potentiometer when the resistance is 11 **Ω**. Without algae present, the measured time constant is roughly 0.5us. This is to be expected since the additional resistance of the algae is not present. With algae and the chamber present, the measured time constant is approximately 1.25us. Using this value as the experimental value, and the measured time constant in simulation, 1.10us, as the accepted value, the percent error is 11.6%. This percent error is acceptable since the time constant is between 1us-2us.

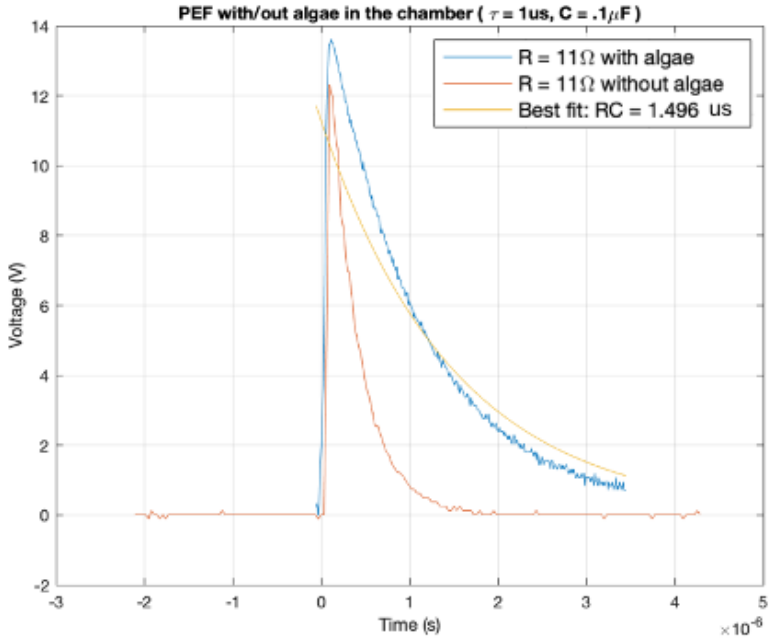

FIGURE 20: 1US WAVEFORM MEASURED ACROSS POTENTIOMETER WITH AND WITHOUT ALGAE CHAMBER

Fig. 21 compares the expected 1us, 3us, and 10us time constant measurements taken with and without algae. The 10us response with algae appears to have a lot of noise. It is speculated that this noise occurs due to the connection cables between the electroporator system and the algae chamber. The 3us and 10us response without algae do not show a complete decaying waveform since the resistance of the algae is not present.

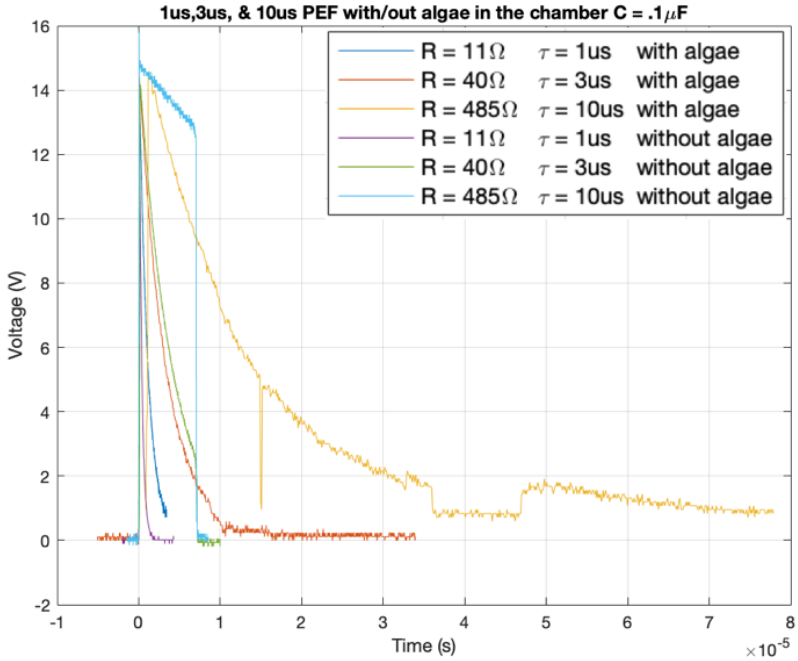

FIGURE 21: *1US, 3US, AND 10US WAVEFORMS MEASURED ACROSS POTENTIOMETER WITH AND WITHOUT ALGAE CHAMBER*

High voltage testing with algae was taken at an expected time constant of 3us and a voltage range of 15V-140V for comparison, which is shown in **Fig. 22**. Most measured time constants from the data below fall within the 2us-3us range. These discrepancies are acceptable since we are hoping to achieve time constants in the 1us-10us range. The discrepancies in the R = 485 **Ω** may be due to inductance created by the amplifier, wires, or solder connections. More testing needs to be conducted on the discontinuities of the waveforms once in person meetings resume. Additional data can be viewed in Appendix A.

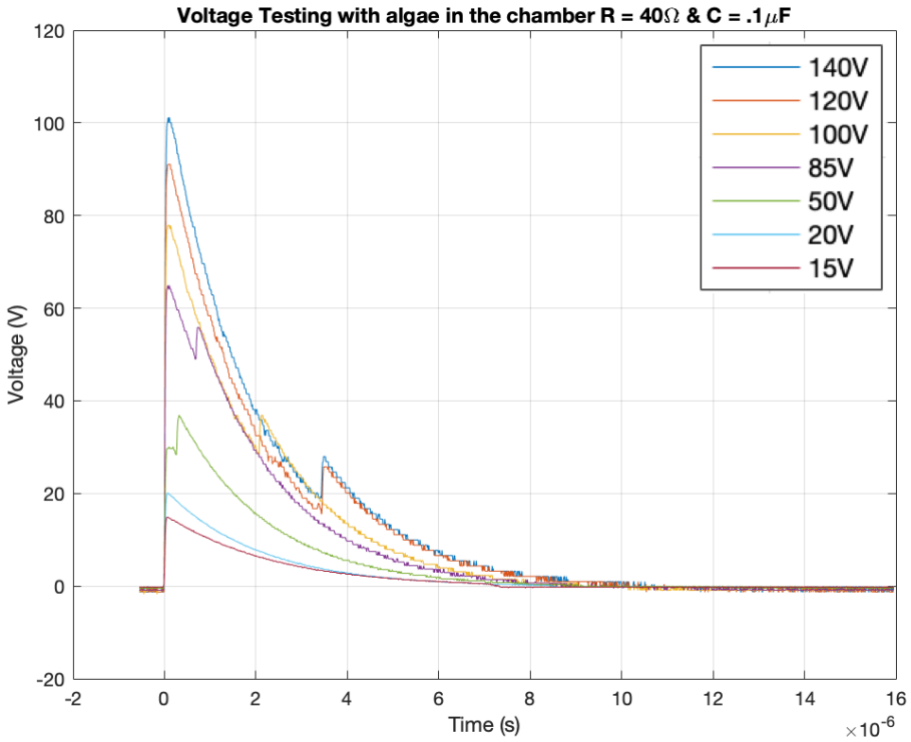

FIGURE 22: 3US WAVEFORM MEASURED ACROSS POTENTIOMETER WITH AND WITHOUT ALGAE CHAMBER

The second pulse generator is constructed with two solid state relays (SSRs) controlled by an Arduino, **Fig. 23**. The Arduino code determines the pulse widths and allows the injection of a train of pulses into the algae. The SSR configuration currently contains two problems. One of the bugs may originate from the predicted ITO + algae impedance model, **Fig. 9**. This conclusion is based on the pulse shape characteristics obtained from modeling the ITO + algae impedance in the mechanical relay and calculating an algae resistance of about 126 **Ω**. The predicted model, shown in **Fig. 9**, would assume an open circuit with DC voltage. The second bug creates an 80% voltage drop across the algae. The electrical team believes that the opto-TRIACs voltage drop and the ITO + algae impedance model causes this voltage drop. The BBM solution was disregarded as the cause of this discrepancy because tests were performed using different solutions to observe how the pulse characteristics change. The test included centrifuging a culture of algae from the bottom of a centrifuge tube, extracting the BBM, re-submerging the algae in deionized water (less conductive that the BBM media solution), and injecting a PEF across the algae. These results concluded that the BBM solution does not alter the pulse shape.

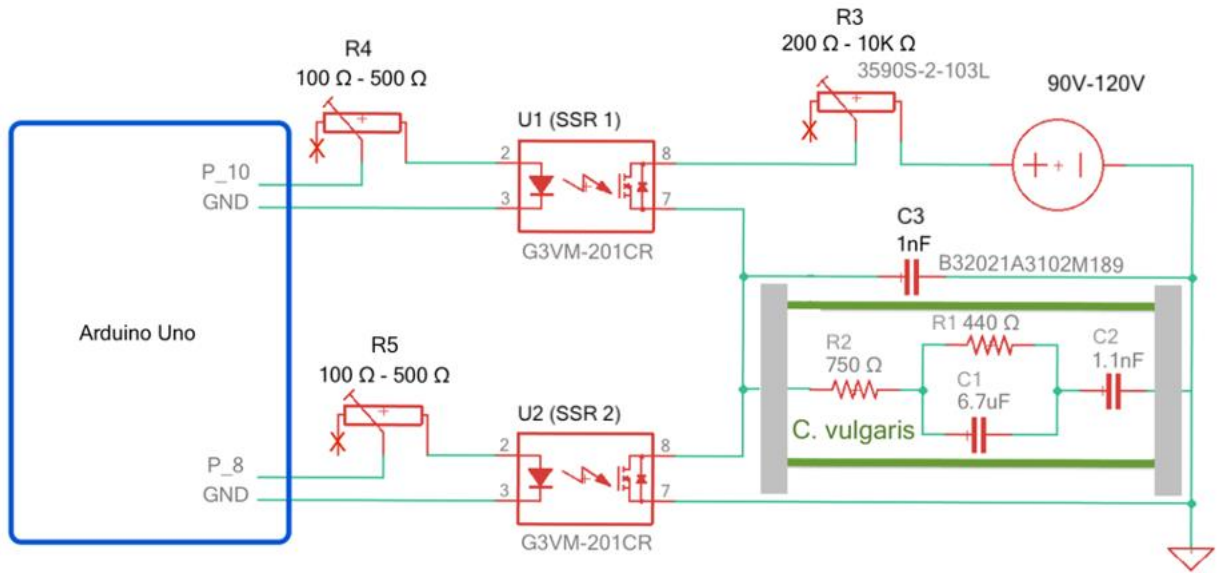

FIGURE 23: SOLID STATE RELAY ELECTROPORATOR CIRCUIT DIAGRAM

TABLE 3
SOLID STATE ALGAE ELECTROPORATOR SSR TRUTH TABLE

Solid State Relay 1	Solid State Relay 2	Circuit State	
ON	OFF	1	Capacitor Charges
OFF	OFF	2	Capacitor discharges into algae chamber
OFF	ON	3	SSR 2 discharges the capacitor
ON	ON	4	Not allowed

The Arduino (5 VDC) supplies the 13mA of input current to turn on the SSRs. The potentiometers control the optocoupler's current. This current controls the light intensity that turns on the transistor and allows current to flow from U1 (SSR 1) pin 8 to pin 7. The logic states of SSR and SSR2 are defined in Table 3. The electroporator system can be seen in **Fig. 24-25**.

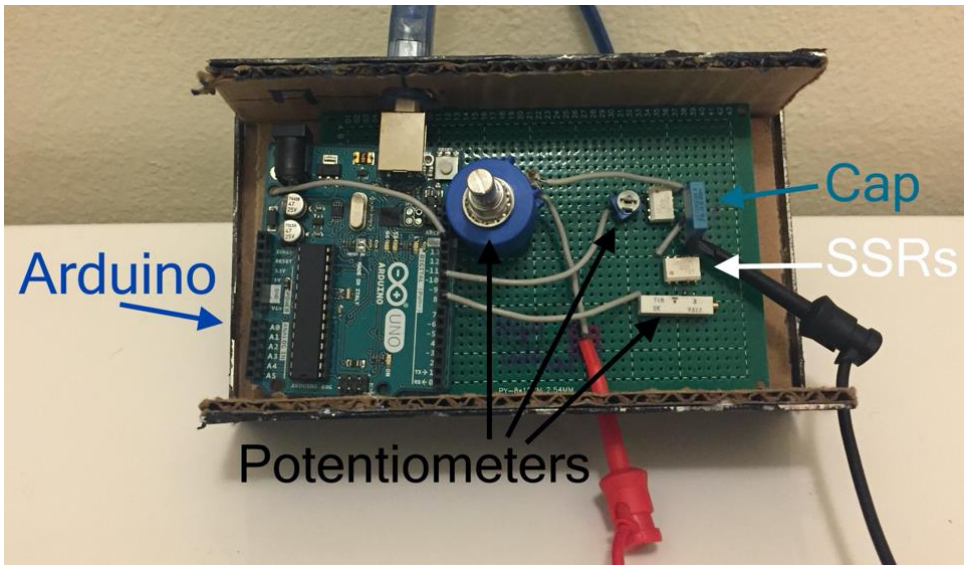

FIGURE 24: SOLID STATE RELAY ELECTROPORATOR COMPONENTS

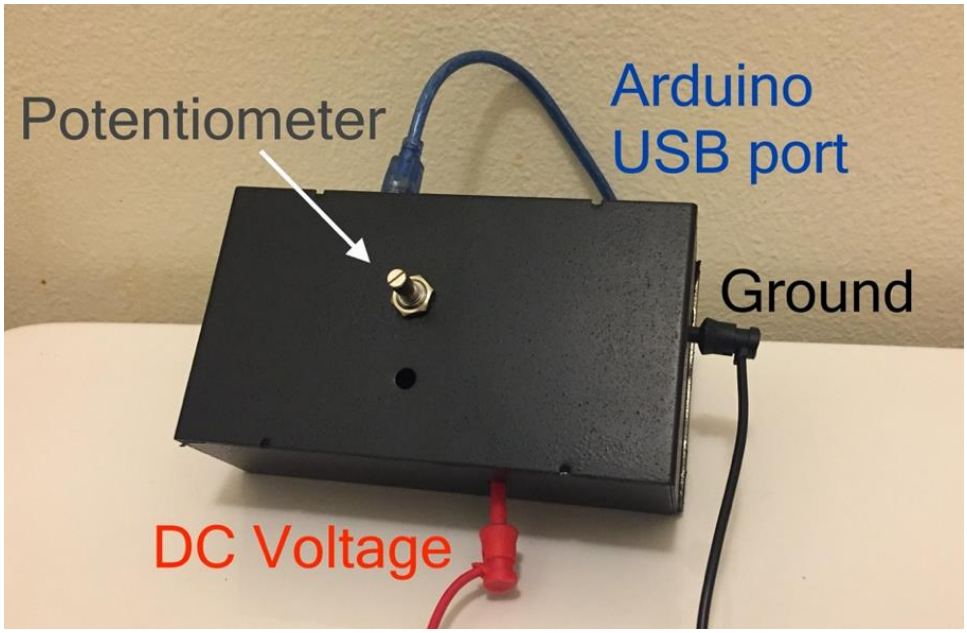

FIGURE 25: ELECTROPORATOR HARDWARE ENCLOSURE

Solid State Relay Electroporator Simulation

The SSR electroporator is simulated using LTSpice. The constructed circuit is in **Fig. 26** and **Fig. 27.** LTSpice does not have a SSR component so two switches are used to mimic the behavior of a relay. The switch 1 functions as SSR1 and switch 2 functions as SSR2 from **Fig. 23.** SSR2 is used to discharge the capacitor node to ground as observed on the right of **Fig. 26**. This truncation time determines the pulse width. If SSR2 truncates the capacitor node at a later time, the pulse width increases.

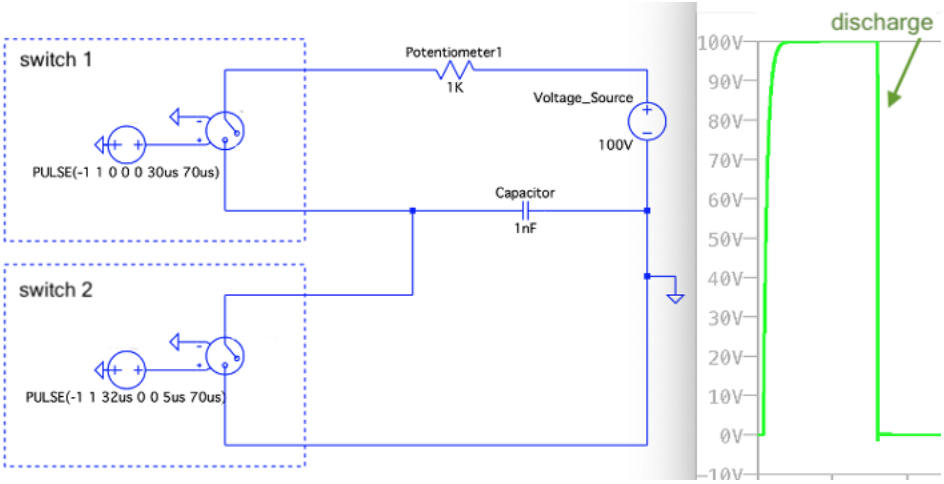

FIGURE 26: LTSPICE ALGAE ELECTROPORATOR SSR CONFIGURATION WITHOUT ALGAE SIMULATION

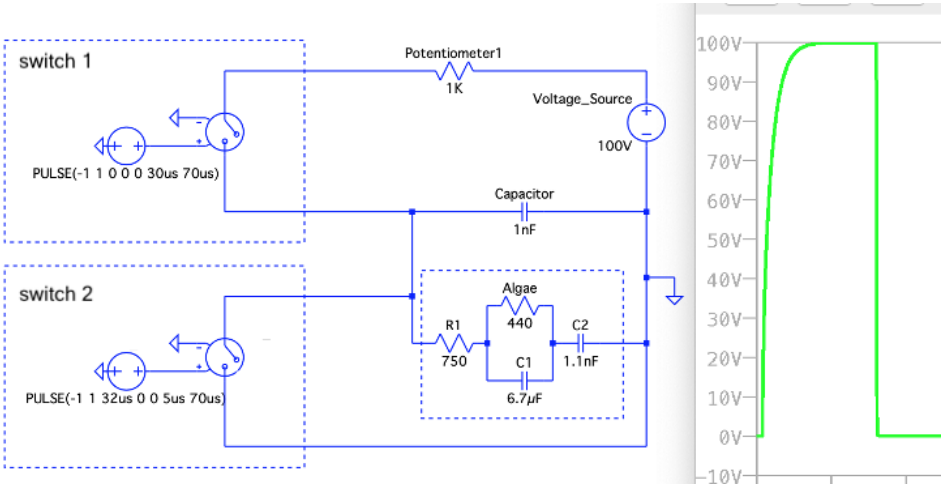

FIGURE 27: LTSPICE ALGAE ELECTROPORATOR SSR CONFIGURATION WITH ALGAE SIMULATION

Solid State Electroporator Data

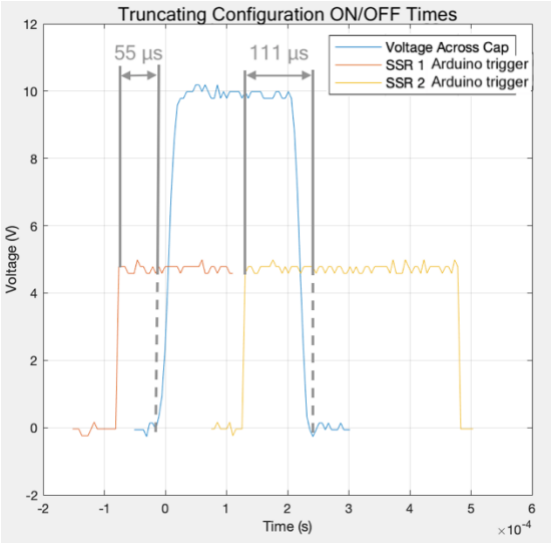

FIGURE 28A: SOLID STATE RELAY PULSE CHARACTERISTICS WITHOUT ALGAE CHAMBER

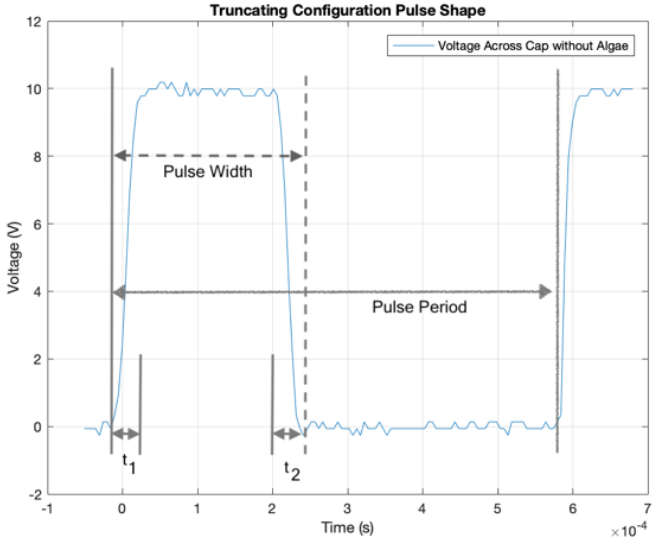

FIGURE 28B: SOLID STATE RELAY PULSE CHARACTERISTICS WITHOUT ALGAE CHAMBER

Fig. 28a depicts the 55 us the Arduino command, digitalWrite, takes to send an input current to the optocoupler and turn-on the transistor. The transistor allows the current to charge the capacitor. The Arduino command takes 111 us to turn-off the transistor. The shape of the electroporator output pulse is shown as the system states transition, as referenced in Table 3. **Fig. 28b** shows the SSR's non-instantaneous rise (t1) and fall time (t2). These rise and fall times were not consistent through different pulse widths and periods.

The following code respectively generates a minimum pulse width of about 30µs and a minimum period of 476µs. This code alters the pulse width and period with the help of 3 delay commands (Delay 1, 2, and 3). To generate a train of pulses the following code is ran in a loop. **Fig. 29** depicts how applying changes to the code alters the pulse shape characteristics. The full Arduino code and algae PEF treatment setup are in Appendix B.

```
// Single Pulse Generation Code
    digitalWrite(SSR1, HIGH);
    delayMicroseconds(100); // Delay 1 - min 100us
    digitalWrite(SSR1, LOW);
    digitalWrite(SSR2, HIGH);
    delayMicroseconds(350); // Delay 2 - min 350us
    digitalWrite(SSR2, LOW);
    delayMicroseconds(5); // Delay 3 - min 5us
```

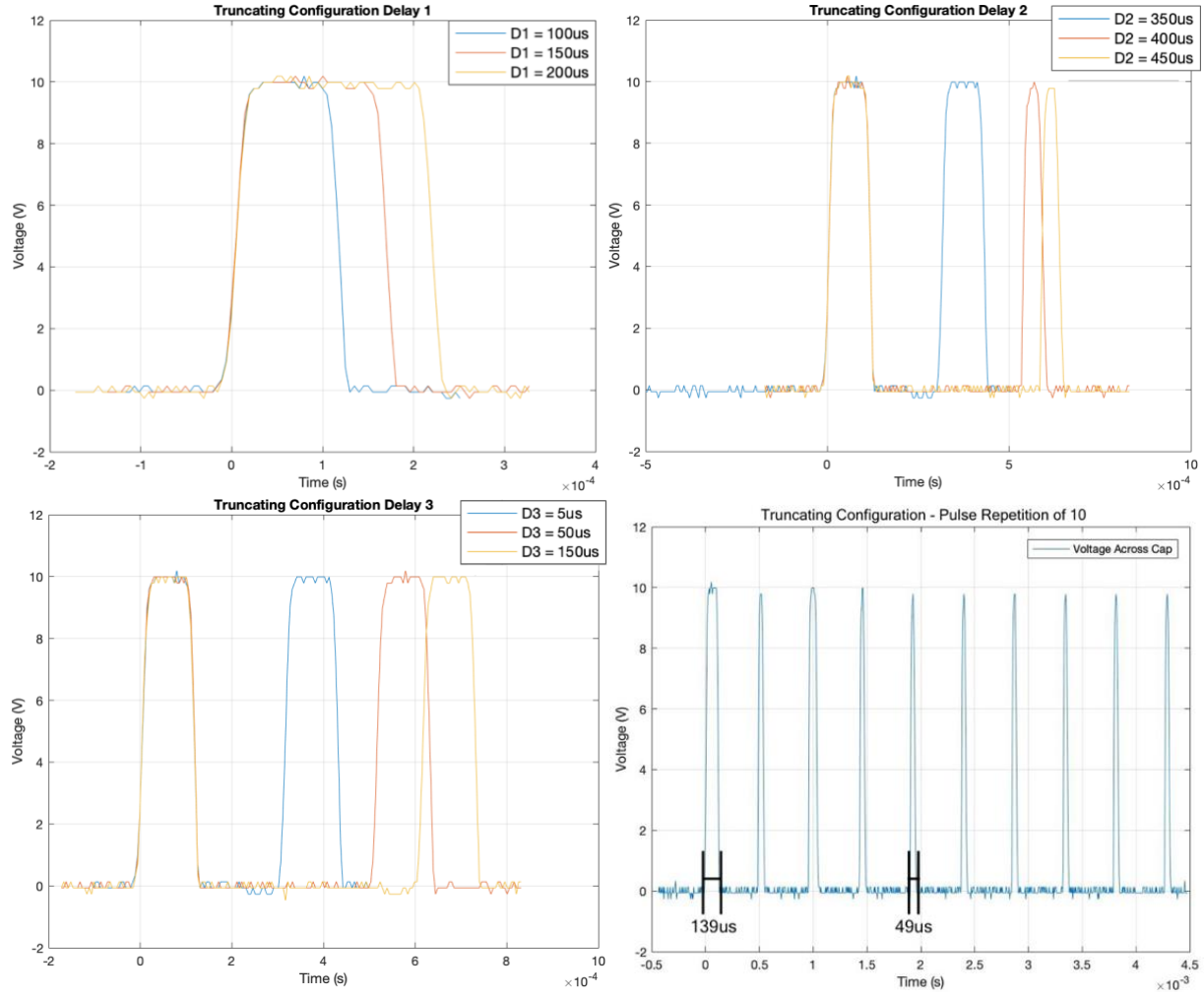

FIGURE 29: SOLID STATE RELAY PULSES CREATED BY CHANGING DELAY TIMES WITHOUT ALGAE CHAMBER

TABLE 4
MECHANICAL RELAY ELECTROPORATOR AND SSR ELECTROPORATOR PARTS LIST

Part Number	**Description**	**Distributor**	**Quantity**	**Total Cost**
495-4962-1-ND	1nF Capacitor	Digi Key	10	$5.08
Unknown	1 kΩ Resistor	-	2	$0.00*
Unknown	3 kΩ Resistor	-	1	$0.00*
Unknown	Mechanical Relay	-	1	$0.00*
Arduino Uno	Micro Controller	-	1	$0.00*
Z8463-ND	Solid State Relay	Digi Key	2	$18.82
Unknown	Potentiometer	-	2	$0.00*
3590S-2-103L	Potentiometer	Allied	1	$13.13
			Total	$37.03

*Parts were donated by various sources in the Cal Poly Electrical Engineering Department or we previously had.

Conclusion

When the research team can reassemble on campus, testing will continue with the mechanical electroporator system. The Biology team will determine if lysis has occurred. The next step of testing with the mechanical electroporator involves sending multiple pulses at least 1s apart to account for the 0.5s relay switching time. Measurements and photos will be taken to determine which voltage, time constant, and amount of pulses yields the best result for lysis of the algae cells.

Modifications may need to be added if it is determined lysis will be achieved by applying multiple pulses to the algae in a time span shorter than 1s. One suggestion to achieve multiple pulses is to introduce additional relays in parallel that can each be triggered separately. This will allow for multiple pulses to be sent into the algae sample to expose it to an electromagnetic field for a longer portion of time than 1us-10us, a possible schematic is depicted in **Fig 30**. Additional modifications that can be made is to change the value of the capacitor. Changing the capacitor value and using the respective potentiometer values in Table 2 will allow a wider range of testing.

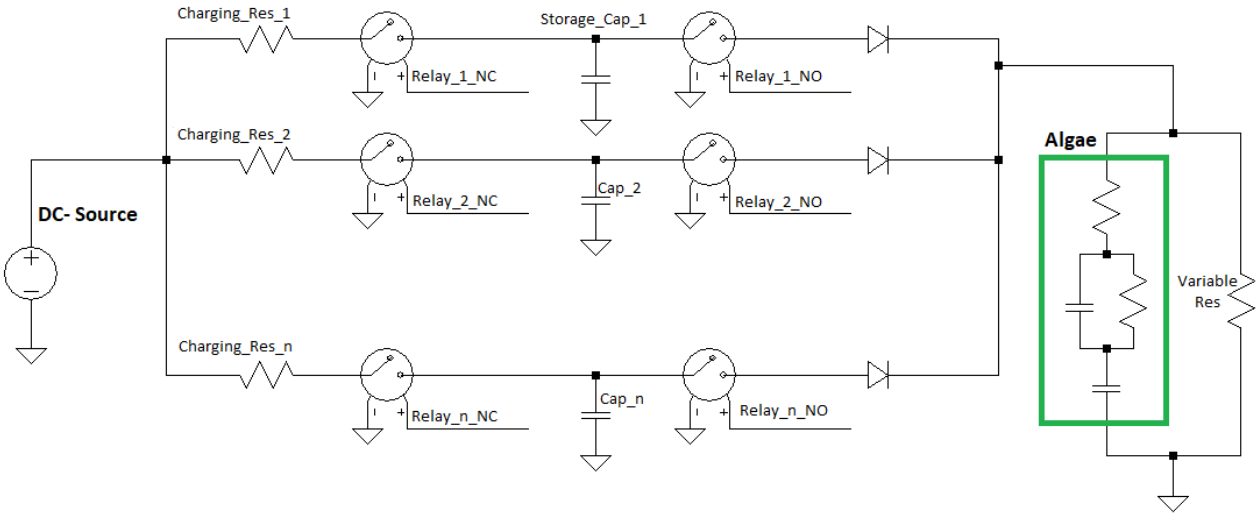

FIGURE 30: PULSE TRAIN ELECTROPORATOR, MULTIPLE MECHANICAL RELAYS

Once testing has commenced on the mechanical relay electroporator and the team has concluded to have a working model, debugging the solid-state relay circuit will begin. The initial discrepancy the team will aim to solve will be the 80% decrease in voltage that is seen across the sample. The team will begin to debug and determine if the voltage measured across each component is as expected. A possible solution is to change the capacitor to 0.1uF and model the ITO + algae impedance with a resistance of 126 Ω. Once this data has been obtained, it will determine the best way to move forward by either replacing components or trying a new circuit configuration.

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Appendix A: Mechanical Relay Electroporator Data

Simulation

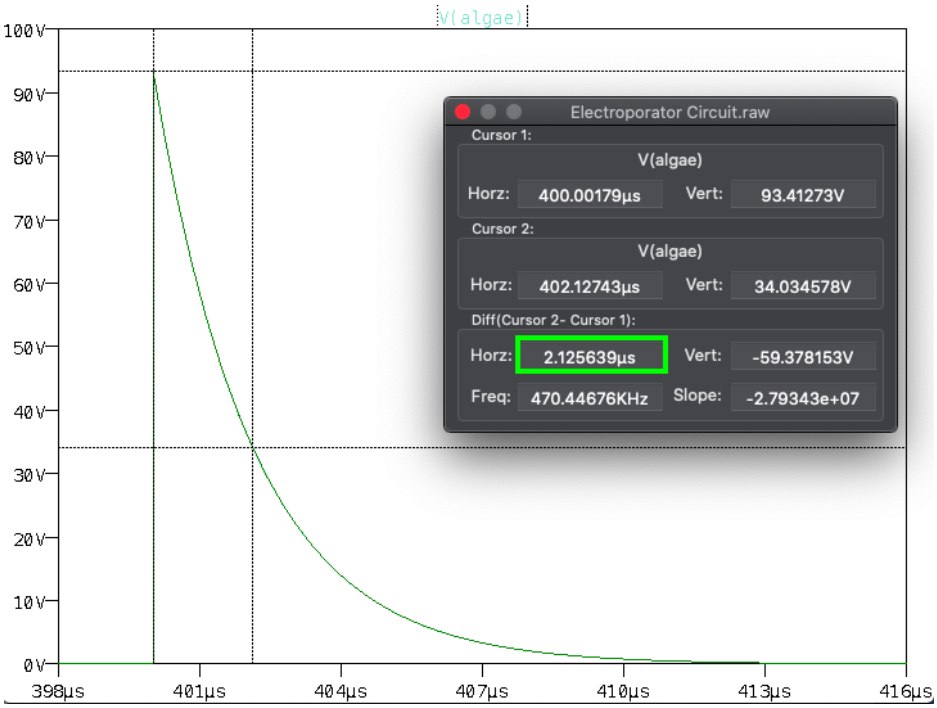

FIGURE 31: 2US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

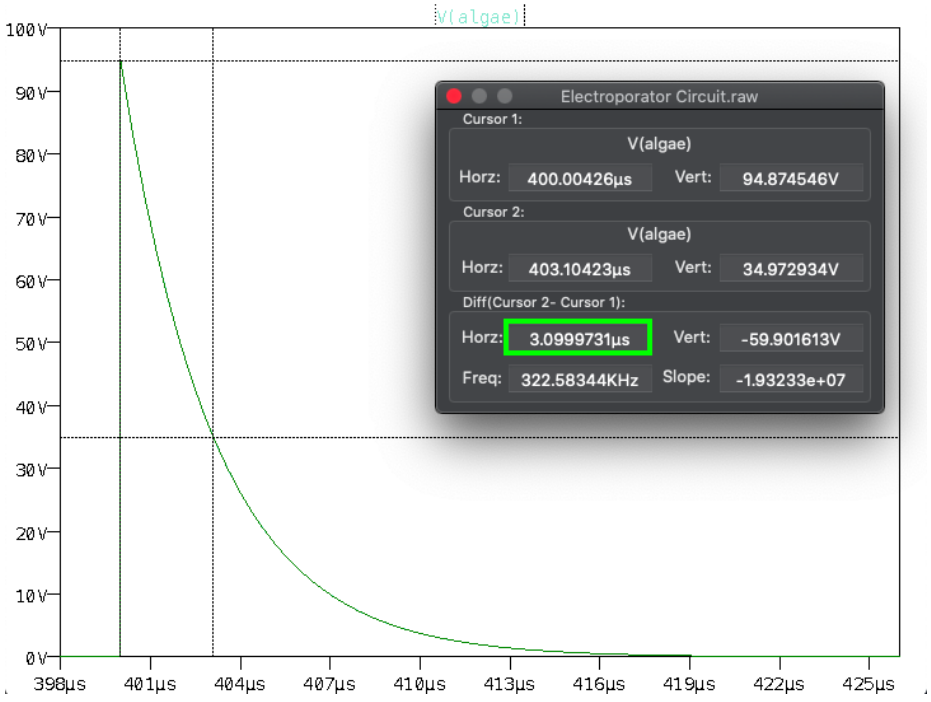

FIGURE 32: 3US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

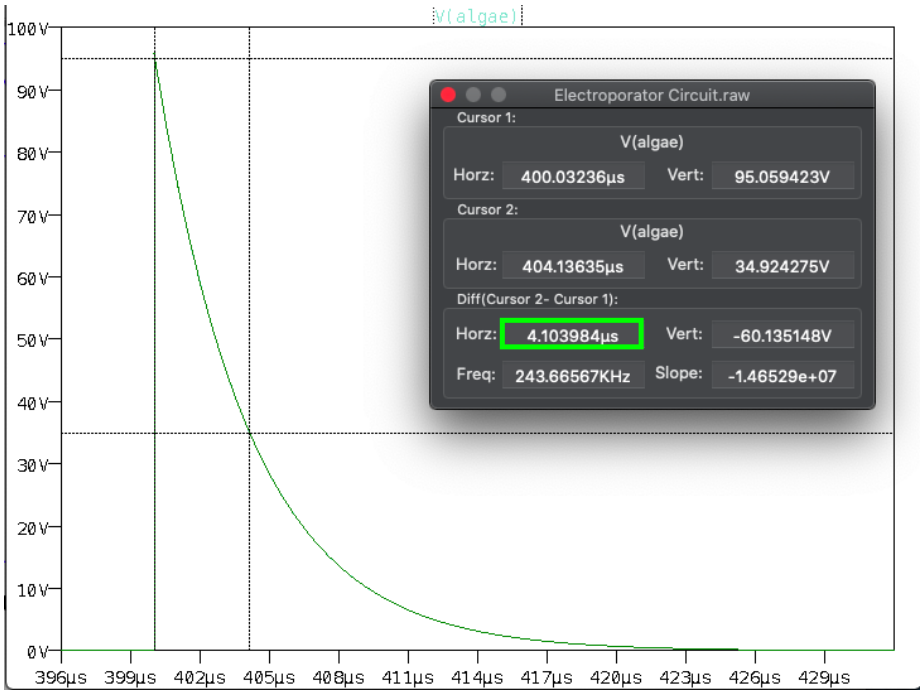

FIGURE 33: 4US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

FIGURE 34: 6US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

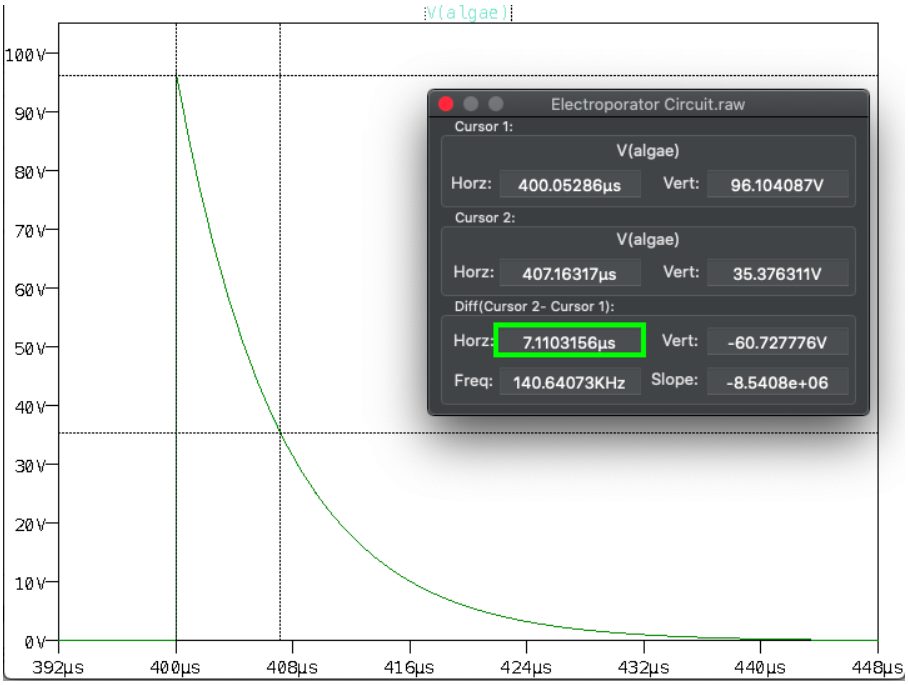

FIGURE 35: 7US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

FIGURE 36: 8US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

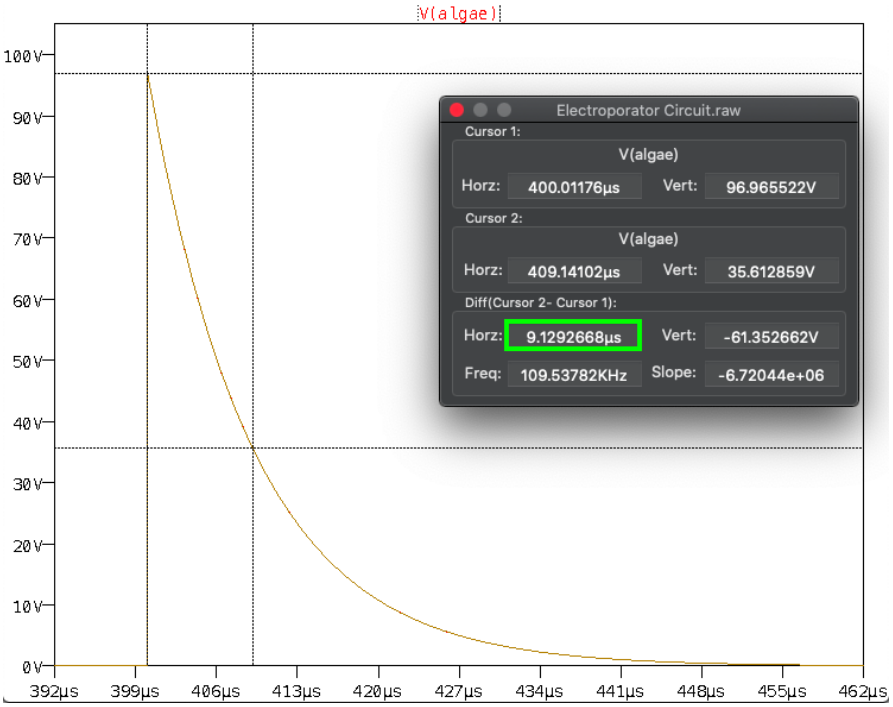

FIGURE 37: 9US WAVEFORM MEASURED ACROSS THE SIMULATED ALGAE

Hardware

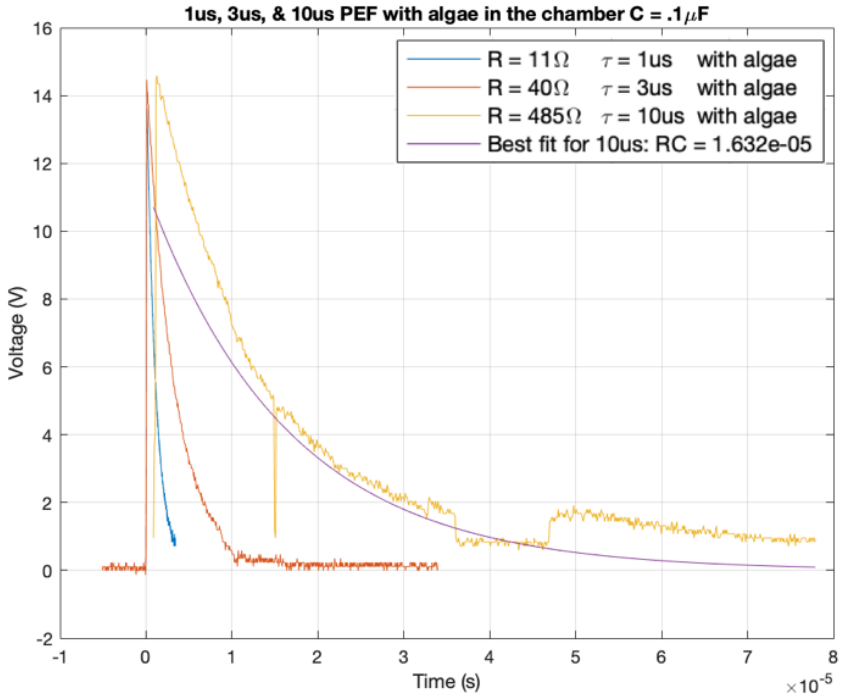

FIGURE 38: LOW VOLTAGE 3US WAVEFORM MEASURED ACROSS POTENTIOMETER WITH ALGAE

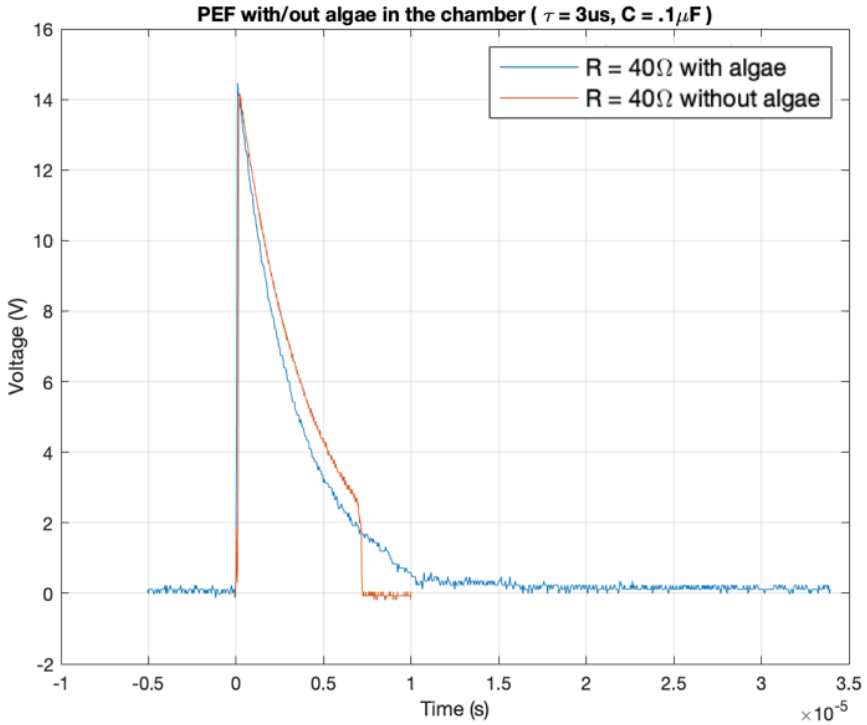

FIGURE 39: LOW VOLTAGE 3US WAVEFORM MEASURED ACROSS POTENTIOMETER WITH AND WITHOUT ALGAE

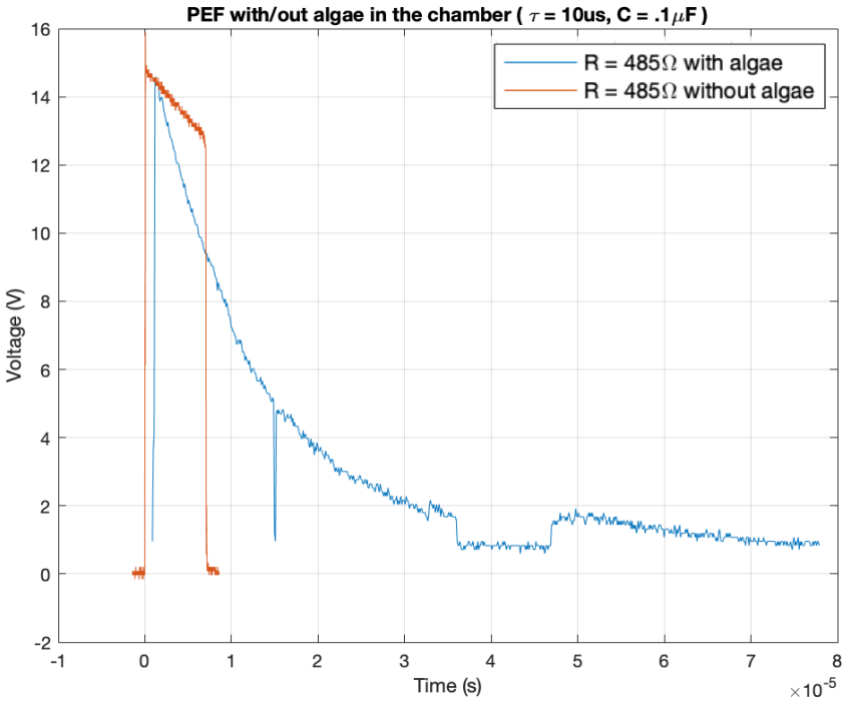

FIGURE 40: LOW VOLTAGE 10US WAVEFORM MEASURED ACROSS POTENTIOMETER WITH AND WITHOUT ALGAE

Appendix B: PEF Treatment Set Up Instructions

PEF Treatment Instructions

A PEF treatment is created by clamping the two ITO conductive plates, shown in **Figure 34**, parallel to each other. The two conductive faces of the ITO glass slides must face one another.

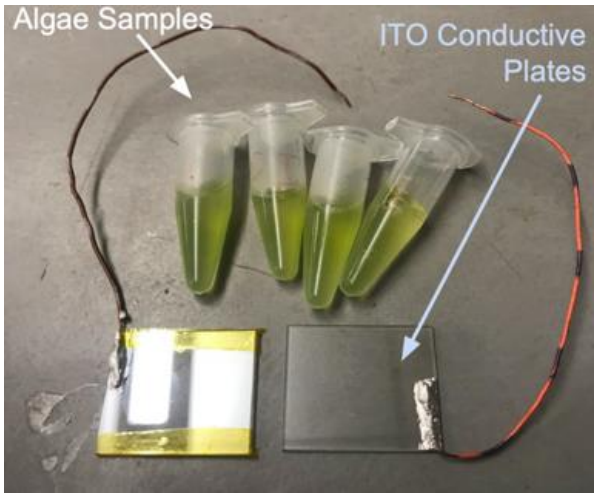

FIGURE 41: *ITO PEF Treatment Chamber*

- With a pipet, place a 0.5mL sample of algae on one side of the chamber and allow capillary action to draw the algae into the chamber.
- Place the ITO PEF chamber under a microscope and locate the algae for viewing
- Connect the cables soldered to the ITO glasses across the capacitor
    - Mechanical Relay Electroporator - **Figure 14**
    - Solid State Relay Electroporator - **Figure 24**
- For the SSR configuration utilize the following Arduino code to create the desired pulse characteristics
    - Delay 1 – Controls Pulse Width (**Figure 29**)
    - Delay 2 – Controls Pulse Period (**Figure 29**)
    - Delay 3 – Controls Pulse Period (**Figure 29**)

In the setup, a DC voltage supply feeds a DC voltage amplifier providing 80V-140V to the electroporator, **Figure 11**. Setting the horizontal time scale on an oscilloscope to 1ms per division and enabling the trigger function will capture the pulse when probed across the capacitor, **Figures 14** and **Figure 24**.

Note(s) :
Record data, capture waveforms, and analyze the circuit through an oscilloscope procedure:

- Temporarily disconnect the ITO PEF (Indium Tin Oxide Pulsed Electric Field) chamber
- Command the Arduino to output an infinite pulse train through a loop function
- Running an infinite pulse train makes it easy to auto scale, set voltage triggers, etc.
- Set the oscilloscope functions as appropriate and command the Arduino to stop
- Reconnect the ITO PEF chamber and manually command the desired PEF treatment

PEF Treatment SSR Electroporator Configuration Arduino Code

```
// Map the solid state relays to an Arduino Pin
  const int SSR1 = 10;
  const int SSR2 = 8;
// Select Pulse Repetition
  int pulse_repetition = 1;

  void setup() {
// Arduino initialization of Solid State Relays
    pinMode(SSR1, OUTPUT);
    digitalWrite(SSR1, LOW);
    pinMode(SSR2, OUTPUT);
    digitalWrite(SSR2, LOW);
  }
  void loop() {
    while(pulse_repetition > 0) {
// Commands to create a single pulse
// [ Delay_1 = 100us, Delay_2 = 350us, and Delay_3 = 5us
// creates a the minimum pulse width of ~30us and a period of 480µs ]
      digitalWrite(SSR1, HIGH);
      delayMicroseconds(400); // Delay 1 - min 100us
      digitalWrite(SSR1, LOW);
      digitalWrite(SSR2, HIGH);
      delayMicroseconds(1000); // Delay 2 - min 350us
      digitalWrite(SSR2, LOW);
      delayMicroseconds(5); // Delay 3 - min 5us
// ...
// Commands to create the nth pulse
      digitalWrite(SSR1, HIGH);
      delayMicroseconds(300);
      digitalWrite(SSR1, LOW);
      digitalWrite(SSR2, HIGH);
      delayMicroseconds(1000);
      digitalWrite(SSR2, LOW);
      delayMicroseconds(5);
    pulse_repetition = pulse_repetition - 1; }
  }
```

Appendix C: Senior Project Analysis

Project Title: Pulsed Electric Field System Development for Algae Biofuel Extraction
Student's Name(s): Aspyn Bessler and Juan David Gonzalez Aguayo
Student's Signature(s):

Advisor's Name(s): Professor Dean Arakaki
Advisor's Signature(s):

The following is holistic analysis that describes the effects, costs, and benefits associated to the manufacturing of the described algae electroporators.

1. Summary of Functional Requirements

The described algae electroporators are designed to administer an electrical pulse to algae cells to attempt irreversible pore formation to extract the cell's lipids.

- The mechanical relay electroporator circuit design contains relays, capacitors, and resistors to outputs PEFs between 1µs to 10µs. The user can select the pulse duration by changing the value of the potentiometer. The system utilizes a relay to switch and discharge a capacitor to the algae sample, creating a pulse electromagnetic field.
- The solid state electroporator circuit design contains solid state relays, an Arduino, a capacitor, and potentiometers to output PEFs up to a 30µs pulse width and a period of up to 476µs. This electroporator turns on one of the SSRs to create a voltage potential across the algae and discharges this voltage potential when the code commands. The Arduino enables controllable pulse widths, periods, and cycles of repetition.

2. Primary Constraints

Delivering PEFs with variable characteristics is the main constraint of this project. These constraints are critical because they limit PEF widths, duty-cycles, period, amplitude, and cycle repetition. A broad range of output PEF characteristics is important to research the attributes that cause membrane rupture. The biggest limiting factor is a pulse shape alteration when algae is introduced into the chamber. The algae itself is believed to cause this pulse mutation as our equivalent circuit model is not 100% accurate.

- The mechanical relay electroporator limiting factors include:
    - The relays time delay between successive repetitions (~500ms)
    - Exclusive discharging capacitor pulse shape
- The solid state electroporator limiting factors include:
    - SSRs turn ON/OFF times constrain the pulse width to no less than 30µs
    - Exclusive square pulse shape

3. Economic

Fixed production costs are minimized as much as possible to reflect a donation given by Boeing. The student project budget does not include human, real, natural, and external costs as they require individual analysis. Table 4 depicts a cost breakdown estimate for the production of one electroporator device.

The cost analysis described contains approximate values to produce one system. The total cost to manufacture and maintain the described algae explicit electroporator depends mostly on its development, forecasted production, and expected device usage.

Note that development and production costs carry less approximation error due to their nature but, device usage costs depend on the device expected usage and reliability. The electroporator requires electrical energy (from a commercial power source), a PEF chamber, algae and BBM, and a way to check lysis for its intended usage.

- The *human capital constitutes* student researchers and project advisors. Externalities such as outsourced manufacturing and component acquisition might also require human capital but are not considered due to their nature.
- The *financial capital includes* test equipment, facility expenses, software licensing, outsourced published scientific literature, algae cultivation materials, prototype construction, and device production.
- The *real capital illustrates* a fraction of the human capital costs because this analysis describes an electroporator constructed manually.
- The *natural capital required* for testing and production include algae, water, facility property, and external resources such as the ones used to generate the electrical energy demanded, and elements used to construct the device components.

4. If Manufactured on a Commercial Basis

The cost analysis disregards both electroporator and biofuel mass-production, and instead derives an approximated analysis based exclusively from manufacturing one device. Electroporator mass-production and market values are approximated and analyzed utilizing Table 4.

- Estimated number of devices sold per year: 20
- Estimated manufacturing cost for each device: $250
- Estimated purchase price for each device: $600
- Estimated profit per year: $7,000

5. Environmental

The project harms algae in the process. The project entails irreversible pore formation of algae to release their stored lipids for collection. Environmental impacts occur when parts of the device are not recycled with care at the end of the electroporator's lifetime.

Electroporator mass-production breakdown:

Positive:

- Electroporators with different characteristics already exist. One can take the environmental impacts associated with their manufacturing and minimize algae electroporator impacts.

Negative:

- Production requires electrical energy obtained from non-sustainable sources
- Facility requires real estate, construction elements, equipment, and maintenance
- Electroporator requires outsourced components
- Electroporator requires proper disposal after its lifetime
- A fraction of the components required for its construction are not environmentally friendly

6. Manufacturability

A challenge that may arise when manufacturing the system is how to mass produce the system in a timely and efficient manner. The initial device is used by Cal Poly biology, electrical engineering, and physics students and staff to conduct research and use the algae's lipids to research biofuel. Improvements to the device are also suggested before considering mass production.

7. Sustainability

The goal of the project depicts creating a device that can administer PEF treatments to algae samples. The electroporator must assist in the processes of creating renewable fuel. The hope is that the algae samples absorb the amount of CO_2 during their growth period to cancel out the CO_2 released into the atmosphere when the biofuel created is used. The electroporator demands electrical energy (from a commercial power source), a PEF chamber, and algae with BBM for its intended usage. It also demands additional facility electrical energy usage and real estate demands when device is mass-produced. Technological, production, and sustainable advancements lead to positive improvements. Additional social and socio-economic improvements appear when the device emerges for consumer usage.

The sustainable practices proceeding the designing of this device follow:

Environmental

- Minimize energy consumed by electroporation
    - Energy efficient switching, and display circuitry
    - Reduction of components
- Salvaged electrical components

Economical

- Cut monetary resources
    - Salvaged electrical components
- Utilized Cal Poly SLO Resources
    - Library
    - Software
    - Laboratories

Social

- Utilize informed sources and experts when designing the device

8. Ethical

TABLE 5 (PART1): BREAKDOWN OF ETHICAL IMPLICATIONS PROCEEDING ALGAE ELECTROPORATION TREATMENT AND ALGAE ELECTROPORATOR DEVICES

Ethical Concerns (Stages of Implication)	**Ethical Analysis Framework**	**Justification &/or Notes**
Algae are living organisms and this device explains a treatment that kills the algae *(Design, Operating, and Developmental Stages)*	Egoism	Algae cell biofuel production promises economical and power acquisition. All organisms directly or indirectly affected when researching must be considered when designing and operating electroporators. The average algae (depending on species) only survive for a few days to two years. The lysing of the algae premature to natural cell death will not drastically alter the lifespan of the living organism.
	IEEE	This device kills the algae cell whereas other electroporation treatments usually disturb the living organisms. The lifespan of the living organisms requires consideration and study. Apoptosis, or cell death, means that biofuel components are deteriorating but, to obtain adequate intracellular components they need to bed accessed early in the algae lifespan.
Production whether large or small scale can cause negative ecological footprints *(Manufacturing, Operating, and Developmental Stages)*	Contractarianism	Rhetorical issues manifest when the message is not clearly defined for its intention. Large scale production of the electroporator or biofuel will cause impacts which are briefly discussed throughout Appendix A. The negative and positive ecological impacts of a unit are discussed in the environmental subsection.
	IEEE	A perfectly sustainable product that does not negatively impact the environment in any manner is not yet fabricated but current sustainable practices maximize positive economic, social, and ecological impacts. Decreasing the fossil fuel dependency while utilizing a fraction of the resources that subsist from fossil fuel refinement presents positive ecological effects.

TABLE 5 (PART 2): BREAKDOWN OF ETHICAL IMPLICATIONS PROCEEDING ALGAE ELECTROPORATION TREATMENT AND ALGAE ELECTROPORATOR DEVICES

Ethical Concerns (Stages of Implication)	**Ethical Concerns (Stages of Implication)**	**Ethical Concerns (Stages of Implication)**
Fossil fuel and biofuel industry changes Biofuel regulations and policies are not resolved and implemented	Utilitarianism Kant's Categorical Imperative	Less fuel emission, increased usage of sustainable energy sources, and positive ecological footprint effects represent global-scale-impacts for the greatest number of living creatures. These impacts ripple to fossil fuel and biofuel industries, possibly altering the job responsibilities, knowledge, and skills in these industries. The undeveloped biofuel industry will advance creating jobs in the process but, the possible decrease in fossil fuel usage might bring layoffs. With the decrease of fossil fuel consumption, petroleum could expand to other more environmentally friendly practicalities. Less fossil fuel economical income to governments means less money to invest in other societal priorities. However, production of biofuel is designed sustainable to create revenue. As both of these implications reach a state of equilibrium, they offset each other.
(Manufacturing, Operating, and Developmental Stages)	IEEE	The algae electroporator is designed to allow researchers and professionals to develop current algae electroporation technologies and knowledge. Sustainable engineering entails ethical practices that encourage considering rhetorical context developed from this device.

9. Health and Safety

Development:

The development process (manual construction and testing) implicates following standard high-voltage laboratory safety procedures. [7] [8]

Usage:

The electroporator requires a commercially available power source using 120 V_{AC} from a US wall socket. Enclosing the electrical elements and placing the algae away from the user is the safest way to operate the machine.

Production:

Large scale production of algae electroporators and algae biofuel projects a small deviation of health and safety concerns from those present in current electroporator manufacturing, algae cultivation methods, and biofuel production. [7] [8]

10. Social and Political

The direct and indirect stakeholders are algae and researchers. The project benefits researchers to help them further understand the process and develop techniques for extracting lipids from an algae sample.

Less fuel emission increase usage of sustainable energy sources and positive ecological footprint impacts depict global scale social and political impacts. Proving governments and every living creature as an indirect stakeholder when presenting large scale usage. Political issues arise if the project progresses to mass and consumer level production.

11. Development

The project challenged us to apply knowledge that we have accumulated in our classes at Cal Poly. It helped us to research technology used previously and apply it to our current designs. Literature search has proven helpful for our initial stages in starting the project.