

DESIGN THE CIRCUIT TO GENERATE  
SQUARE WAVE FOR ELECTROPORATION  
TECHNIQUE

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DESIGN THE CIRCUIT TO GENERATE SQUARE WAVE FOR  
ELECTROPORATION TECHNIQUE

SYAMIZA BINTI GHAZALI

Thesis submitted in fulfilment of the requirements  
for the award of the degree of  
Bachelor of Engineering Technology in Electrical

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UNIVERSITI MALAYSIA PAHANG

JANUARY 2018

## STATEMENT OF AWARD FOR DEGREE

### 1. **Bachelor of Engineering Technology**

Thesis submitted in fulfilment of the requirements for the award of the degree of Bachelor of Engineering Technology in Electrical.

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## ABSTRACT

In daily life, fossil fuels is a primary energy sources but it cannot hold for a lifetime. This is because, every day, people use the fossil fuels to generate energy and cause the decrease of the main source of the energy in the future. Besides, burning the fossil fuels also can give bad effect to greenhouse which usually come from transportation and industry. It can cause global warming which can lead to climate changes.

Therefore, our research aimed how to produce lipid that make biodiesel in a short time, with less energy input and eco-friendly. To do this we used electroporation process, where we employed a high voltage for shorter time on yeast cell to disrupt them. Our reactor took around 5 minutes to disrupt the cell wall for escaping intracellular lipid which could be used for biodiesel synthesis. The developed circuit can employ 1KHz to 8KHz. . The circuit can generate square wave output for electroporation process and can control the frequency and voltage as well.



## ABSTRAK

Dalam kehidupan seharian, bahan api fosil adalah sumber utama tenaga tetapi ia tidak boleh tahan untuk seumur hidup. Ini adalah kerana, setiap hari, orang menggunakan api fosil untuk menjana tenaga dan menyebabkan pengurangan sumber tenaga utama pada masa akan datang. Selain itu, pembakaran bahan api fosil yang juga boleh memberikan kesan buruk kepada rumah hijau yang biasanya datang dari industri dan pengangkutan. Ia boleh menyebabkan pemanasan global yang boleh membawa kepada perubahan iklim.

Oleh yang demikian, kajian bertujuan untuk memastikan hasil lipid yang membuat biodiesel dalam masa yang singkat dengan kurang tenaga input dan mesra alam. Untuk melakukan ini kita menggunakan proses electroporation, di mana kita bekerja pada voltan tinggi yang bagi masa yang lebih singkat pada sel yis untuk mengganggu mereka. Reaktor kami mengambil masa kira-kira 5 minit untuk mengganggu dinding sel untuk memecahkan dinding sel yang boleh digunakan untuk sintesis biodiesel. Litar maju boleh menggunakan 1KHz untuk 8KHz. Litar boleh menghasilkan gelombang persegi output bagi proses electroporation dan boleh mengawal frekuensi dan voltan juga.

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**LIST OF SYMBOLS**

V	Volt
A	Ampere
%	Percentage
s	Second
min	Minute
T	Time
EP	Electroporation
f	frequency
kV	Kilo volt

**LIST OF ABBREVIATION**

ARDUI	Arduino
SPDT	Single Pole Double Throw
PWM	Pulse-width modulation
DC	Direct current
AC	Alternating current

## CHAPTER 1

### INTRODUCTION

#### 1.3 BACKGROUND OF STUDY

In daily life, fossil fuels is a primary energy sources but it cannot hold for a lifetime. This is because, every day, people use the fossil fuels to generate energy and cause the decrease of the main source of the energy in the future. Besides, burning the fossil fuels also can give bad effect to greenhouse which usually come from transportation and industry. It can cause global warming which can lead to climate changes.

Electroporation is a membrane phenomenon which involves fundamental behavior of cell and artificial bilayer membranes, and increasingly attracts consideration for applications in biology, biotechnology and medicine. The phenomenon of electropermeabilization of cell membranes has been known for several decades, and has recently received increasing attention for the manipulation of cells and tissues [1-7]. Very early observations suggested that some type of “electrical breakdown” might occur in electrically stimulated membranes [8]

To overcome this scenario, biofuel is one of the sources that can be considered as sustainable solution. Biofuels represent an immense growth opportunity around the world and have an important role to play in displacing the fossil fuels that the world had relied upon it in the past with an idea of more cleaner and renewable alternative. The benefits to the environmental by using biofuel have been widely documented. Biofuels also can provide a new market for the farmer. It can provide an extra dimension for the agricultural sector, providing more demand for the farmers to produce and diversifying the markets, so, they can sell their agricultural products to fulfil their needed and also give new business to our regional communities.

Meanwhile, biofuels can be produce from the organic source that are not limited. From all the alternative energy, biomass is one of the promising sources to produce biofuels. Biomass could provide a huge amount of energy in ways of biofuels and also can offering the world with a new economic market. Biofuels is a liquid fuel which is comes from renewable plants materials. Biofuel is commonly produced from the plant which is through the biological processes.

Electroporation is the action or the process of introducing DNA or chromosomes into bacteria and the other cells using a pulse of electricity to briefly open the pores in the cell membranes. Electroporation is a physical transfection method that practises an electrical pulse to create temporary pores in the cell membranes through a substance like



nuclei acids that can pass into cells. Electroporation is established on a simple process. Host cells and selected molecules are both suspended in a conductive solution, and an electrical circuit is closed around the mixture. An electrical pulse at an optimized voltage can only lasting a few microseconds to a millisecond if discharged through the cell suspension.

The main advantage of electroporation is its applicability for transient and also stable transfection of all cell types. Moreover, because electroporation is easy and rapid, it is able to transfect a large number of cells in a short time once optimum electroporation conditions are determined. The major drawback of electroporation is substantial cell death caused by high voltage pulses and only partially successful membrane repair which requiring the use of greater quantities of cells compared to chemical transfection methods.

The living biomass used carbon dioxide as it grows and then releases back the carbon dioxide when used for energy. This process will result in a carbon-neutral cycle which does not increase the atmospheric concentration of the greenhouse gases. Biomass energy is known to produce electricity, fuels or chemicals. When the biomass is use for this purpose, it is call bioenergy. Biofuels is produced from assorted lignocellulosic materials such as agricultural and forest residues also along with herbaceous materials and urban wastes. This is because the lignocellulosic biomass is one of the most plentiful plants in the world and is a critical feedstock for the manufacture of renewable fuels.

Pretreatment process that is effective should be able to preserve and decrystallize the celluloses and depolymerize hemicelluloses. So, the formation of inhibitors which resist the hydrolysis of carbohydrates should be restrict, low energy input, recovery of value added products such as lignin and also and the cost should be effective. Pretreatment can severely change the properties of the pretreated materials. An effective pretreatment can increase the rate of enzyme hydrolysis and significantly decrease the amount of enzymes needed to convert the biomass into sugars, which can be utilized by microorganisms.

The efficiency of conversion of sugar is influenced by the amount of lignin that are present in pretreatment biomass as lignin is responsible for unproductive binding of enzymes. Removing lignin during the pretreatment process will allow recovery and reuse of enzymes causing in significant cost savings. The obstacles in the existing pretreatment processes also included the insufficient separation of cellulose and lignin. This can reduce the effectiveness of subsequent enzymatic cellulose hydrolysis. The formations of the process can give products that inhibit ethanol fermentation. For example, the acetic acid from hemicellulose, furans from sugars and phenolic compounds from the lignin fraction. High usage of chemicals or energy are consider as waste production and not so friendly to the environment.

## **1.2 PROBLEM STATEMENT**

Though electroporation offer shorter processing time and lower production cost of biodiesel from microbial biomass, still need to be developed some factors to attain potential biodiesel production. Such as:

### **1.2.1 Pretreatment step**

Pretreatment methods of different biomass feedstocks for bioethanol production were intensively investigated. The alternative physical pretreatments of biomass feedstocks include ultrasound, microwave, extrusion, etc. The complexity of lignocellulosic biomass structure is the main obstacle for commercial use. These structural complications are the reason why a pretreatment step is necessary for obtaining fermentable sugars during the hydrolysis step. Although there are many types of pretreatment existed, however they have their own limitation. Besides, biofuels production is mostly oriented with fermentation process, which requires fermentable sugar as nutrient for microbial growth. Therefore, the pretreatment step is necessary for obtaining fermentable sugars during the hydrolysis step.

### **1.2.2 Water content**

the process is not applicable for large-scale biodiesel production having some difficulties such as high water content of biomass that makes the reaction rate slower and hurdles of cell disruption makes the efficiency of oil extraction lower. Additionally, it requires high heating energy in the solvent extraction and recovery stage.

## **1.3 SCOPE OF STUDY**

### **1.3.1 Application of electroporation on Azolla**

To produce third generation biodiesel, microbial oil is considered as a potential source of triglyceride. The most challenging part of this process is the extraction of lipid from microbial cell.

In this treatment, an external electric field is applied to increase cell conductivity and permeability. Electroporation technology enhances the transesterification process, producing pores in the microbial cells by exposing the substrate to electrical fields.

A feasibility study of electroporation as a pretreatment method has been conducted by Garoma and Shackelford (2014) for algal biomass used as feedstock for anaerobic digestion to enhance the production of bio-CH<sub>4</sub>. Our project focused on the application of electroporation on the Azolla cell disruption. Similar to bacteria, the Azolla species have a cell wall that interferes with the transport of molecules to and from the cell.

Azolla cells are destroyed when subjected to high voltage field pulses with sufficient strength and time of treatment. For all microorganisms examined, threshold values of the field strength and the treatment time could be calculated. The cell breakdown is additionally influenced by the relevant cell shape and size.

#### **1.4 OBJECTIVES**

1. To design an electroporation circuit in order to obtain high voltage
2. To fabricate the portable equipment.
3. To deform the cellulosic structure of biomass by electroporation processes.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.0 INTRODUCTION

Electroporation is the use of high-voltage electric shocks to increase biological cell conductivity and permeability because it requires fewer steps, can be easier than alternate techniques. The use of electric fields at certain parameters temporarily permeable the membrane and destroys the cells afterwards exposing certain protein to the surrounding [1]. Electroporation has also been used to extract molecules from cells [2]. Our project focuses on the mainly in irreversible electroporation in which the structure of the cellulose or hemicellulose of lignocellulosic biomass will be disordered or deformed to release fermentable sugar.

Basically, a standard Pulsed Electric Field (PEF) treatment system consists of a pulse generator that enables continuous pulse treatment, flow chambers with electrodes and a fluid-handling system [3]. Electroporator designed for PEF flow application must meet specific and often demanding requirements: high voltage and high current pulses operating in flow-through systems. However, large scale electroporator (PEF systems) require large treatment volumes and have fixed pulse parameters, so they are not useful for investigating the pulse parameter effect in a treatment process [4].

When using a conventional square wave generator, a major limitation is the voltage and current capacity of solid state switches. Switching to voltages of a few kV, despite the advances in the semiconductor technology, is still proving to be problematic. In a series configuration, the load voltage of semiconductors can be reduced but problems can occur because the wiring introduces additional inductance and capacitance in the circuit, which results in voltage spikes [5]. Circuits for the even distribution of voltage in semiconductors connected in series more or less influence the choice of control mode of individual semiconductor devices and it is the easiest if the control semiconductors in series are electrically isolated.

Moreover, snubber or active voltage balance circuits ensure an even voltage distribution in the semiconductors. For switching voltages of up to 4 kV, conventional optocouplers at high voltage can be used; for higher voltages, the choice is among the special optocouplers, optical fibers or transformers[6,7]. Transformers are used for transforming alternating current input voltage into output voltage having coils with a common iron core. Series-connected semiconductors are built with basic building blocks: a semiconductor driving circuit with galvanic insulation, a balance (distribution)

circuit, a protection circuit and a power supply (DC/DC converter). This is actually a basic stack in series.

The influence of pulse duration on the PEF performance has been investigated in previous research. It was shown that PEF process with a shorter duration produce less effective inactivation as compared with the same number of longer impulses [8]. These results are in good agreement with the experimental results reported in [9], which indicated that square waveforms with longest duration produce the strongest inactivation. Therefore, our project is using square waveforms. This is because its control system is simple, the current produced is high, it has a symmetrical shape associated with a perfect square wave and the time parameter is flexible.

Some researchers were conducting a possibility study about electroporation as a pretreatment method that uses algal biomass as feedstock for anaerobic digestion to enhance the production of biogas [10]. Unfortunately, only certain types of cells can withstand the PEF strength. So, a suitable biomass must be chosen thoroughly for the experiments. Cells with a hard cell wall are not effective if using the PEF strength as it will result in biological membrane disruption which causes the release of intracellular compounds [10]. The membrane becomes unstable and breaks because of the formation of pores due to the electrical field. So, potential and suitable materials of lignocellulosic biomass must be used [10]. Hence, for our project we use the Azolla as our testing biomass to investigate the effect of electroporation towards the Azolla.

## 2.1 THEORY OF ELECTROPORATION

Electroporation designates the use of short high-voltage pulses to overcome the barrier of the cell membrane. By applying an external electric field, which just surpasses the capacitance of the cell membrane, transient and reversible breakdown of the membrane can be induced. This transient, permeabilized state can be used to load cells with a variety of different molecules, either through simple diffusion in the case of small molecules, or through electrophoretically driven processes allowing passage through the destabilized membrane.

Electroporation is achieved when the  $DV_m$  superimposed on the resting transmembrane potential is larger than a threshold,  $DV_s$ . As the bilayer membrane is a common feature for eucaryotic cells,  $DV_s$  is similar for various cell types.  $DV_s$  is generally reported to be in the order of 1 V (Kinosita & Tsong 1977a), although an experimental and theoretical study has later described it as being 200 mV (Teissie & Rols 1993).

One class of electroporation theories is based on transient aqueous pores that are explicitly assumed to be created by the combined effects of thermal fluctuations and the local electric field across the membrane. The first version of transient aqueous pore theories was presented by Chizmadzhev and co-workers in a series of seven back-to-back papers, of which only the first is cited here (Abidor et al., 1979).

Optimization of the electroporation process involves several factors. Choosing the wave form, determining field strength and adjusting pulse length are just a few critical variables. Other parameters which play a crucial role in optimization include cell diameter, DNA concentrations, temperature and electroporation buffer

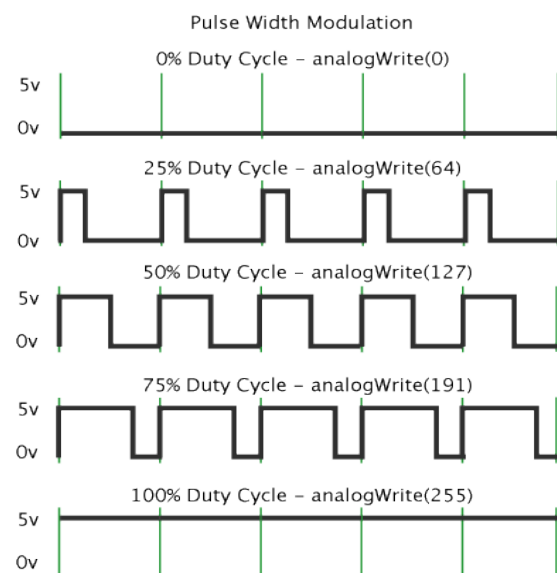
## CHAPTER 3

### METHODOLOGY

#### 3.1 Pulse-width modulation (PWM) with Arduino board

Pulse Width Modulation, or PWM, is a technique for getting analog results with digital means. Digital control is used to create a square wave, a signal switched between on and off. This on-off pattern can simulate voltages in between full on (5 Volts) and off (0 Volts) by changing the portion of the time the signal spends on versus the time that the signal spends off. The duration of "on time" is called the pulse width. To get varying analog values, we are change or modulate that pulse width. We also repeat this on-off pattern fast enough with an LED for example, the result is as if the signal is a steady voltage between 0 and 5v controlling the brightness of the LED.

In the graphic below, the green lines represent a regular time period. This duration or period is the inverse of the PWM frequency. In other words, with Arduino's PWM frequency at about 500Hz, the green lines would measure 2 milliseconds each. A call to `analogWrite()` is on a scale of 0 - 255, such that `analogWrite(255)` requests a 100% duty cycle (always on), and `analogWrite(127)` is a 50% duty cycle (on half the time) for example



**Figure 1 Duty cycle up to 100%**



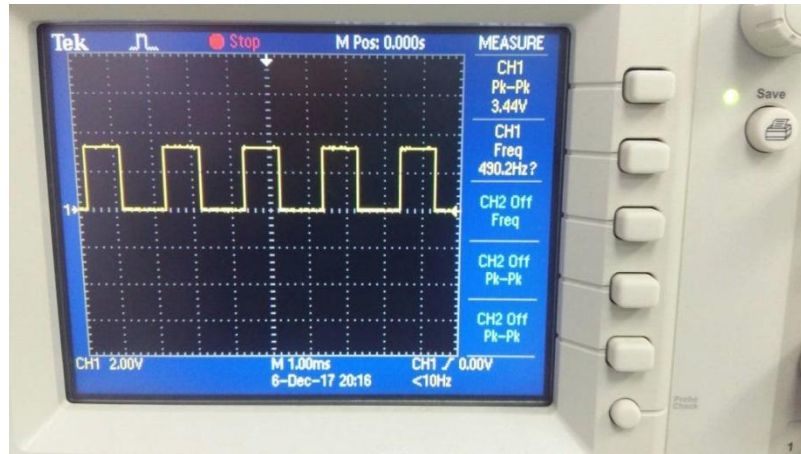


Figure 2 oscilloscope



Figure 3 Arduino board controller

### 3.1.1 Programming the Arduino

```
// include the library code:
#include <LiquidCrystal.h>

// initialize the library with the numbers of the interface pins
LiquidCrystal lcd(12, 11, 5, 4, 3, 2);

const int PWM = 9; // Output to control pulse generator
const int analogInPin = A0; // Analog input pin that the potentiometer is
attached to
const int analogOutPin = 10;
int sensorValue = 0; // value read from the pot
int outputValue = 0;

// the setup function runs once when you press reset or power the board
void setup() {
  Serial.begin(9600);
  pinMode(PWM, OUTPUT);
  // set up the LCD's number of columns and rows:
  lcd.begin(16, 2);
  // Print a message to the LCD.
  lcd.setCursor (0,0);
  lcd.print("frequency= ");
  lcd.print(sensorValue);
  lcd.setCursor (0,1);
  lcd.print("output= ");
  lcd.println(outputValue);
  pinMode(PWM, OUTPUT);
}
// the loop function runs over and over again forever
void loop()
{ analogWrite(analogOutPin, outputValue);
  // print the results to the serial monitor:
  Serial.print("frequency = ");
  Serial.print(sensorValue);
  Serial.print("output= ");
  Serial.println(outputValue);
  // for the analog-to-digital converter to settle
  // after the last reading:
  delay(1000);
  {
    sensorValue = analogRead(analogInPin);
```

```
if(sensorValue < 100)
{
  digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is the
voltage level)
  delay(100);              // use delay to produce 1000Hz
  digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
  delay(100);
}
else if(sensorValue < 200)
{
  digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is the
voltage level)
  delay(100); // produce 2000Hz
  digitalWrite(PWM, LOW); // turn the pulse generator off by making the
voltage LOW
  delay(100);
}
else if(sensorValue < 300)
{
  digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is the
voltage level)
  delay(100); // produce 3000Hz
  digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
  delay(100);
}
else if(sensorValue < 400)
{
  digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is the
voltage level)
  delay(100); // produce 4000Hz
  digitalWrite(PWM, LOW); // turn the pulse generator off by making the
voltage LOW
  delay(100);
}
else if(sensorValue < 500)
{
  digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is
the voltage level)
  delay(100);              // produce 5000Hz
  digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
```

```
    delay(100);
  }
  else if(sensorValue < 600)
  {
    digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is
the voltage level)
    delay(100); // produce 6000Hz
    digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
    delay(100);
  }
  else if(sensorValue < 700)
  {
    digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is
the voltage level)
    delay(100); // produce 7000Hz
    digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
    delay(100);
  }
  else if(sensorValue < 800)
  {
    digitalWrite(PWM, HIGH); // turn the pulse generator on (HIGH is
the voltage level)
    delay(100); // produce 8000Hz
    digitalWrite(PWM, LOW); // turn the pulse generator off by making
the voltage LOW
    delay(100);
  }
  }
}
```

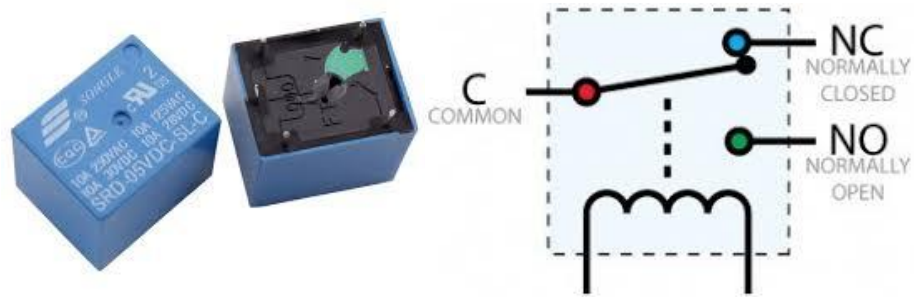
### 3.2 Pulse Generator



Figure 4 pulse generator

Pulse generator which is high voltage output were purchased from local market. Its can produce 8kV and 2-5A output current depends on the load. This generator uses for Tesla coil principle to produce high voltage medium current. The power supply of this generator from 3.7V to 6V red is positive from power source. 6V from lithium battery source with 12Ah capacity was used to ensure a high conductivity.

### 3.3 Relay



**Figure 5 relay**

Relays are switches that open and close circuits electromechanically or electronically. Relays control one electrical circuit by opening and closing contacts in another circuit. As relay diagrams show, when a relay contact is normally open (NO), there is an open contact when the relay is not energized. When a relay contact is Normally Closed (NC), there is a closed contact when the relay is not energized. We use the SPDT relay to act as a control switch in the controller circuit that will turn on and off the pulse generator.

### 3.4 LCD

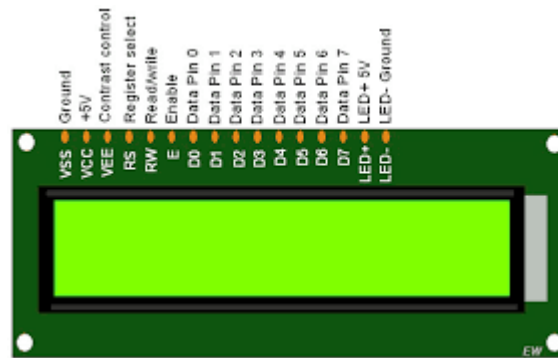


Figure 6 liquid crystal display

LCD (Liquid Crystal Display) screen is an electronic display module and find a wide range of applications. A 16x2 LCD display is very basic module and is very commonly used in various devices and circuits. These modules are preferred over seven segments and other multi segment LEDs. The reasons being: LCDs are economical; easily programmable; have no limitation of displaying special & even custom characters (unlike in seven segments), animations and so on. The command register stores the command instructions given to the LCD. A command is an instruction given to LCD to do a predefined task like initializing it, clearing its screen, setting the cursor position, controlling display etc. The data register stores the data to be displayed on the LCD. The data is the ASCII value of the character to be displayed on the LCD.

### 3.5 6V Lithium Battery



Figure 7 battery

The voltage is 6V and its capacity is 12Ah. The dimension of this battery is 15.1 length x 5 width x 9.8 height in cm. The weight is 1.85kg.



### 3.6 Transistor 2N222

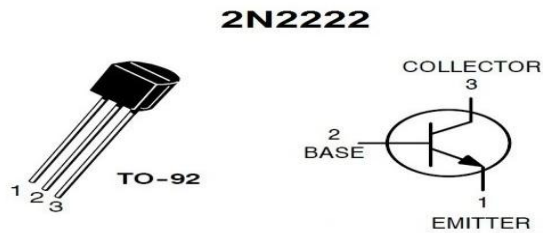
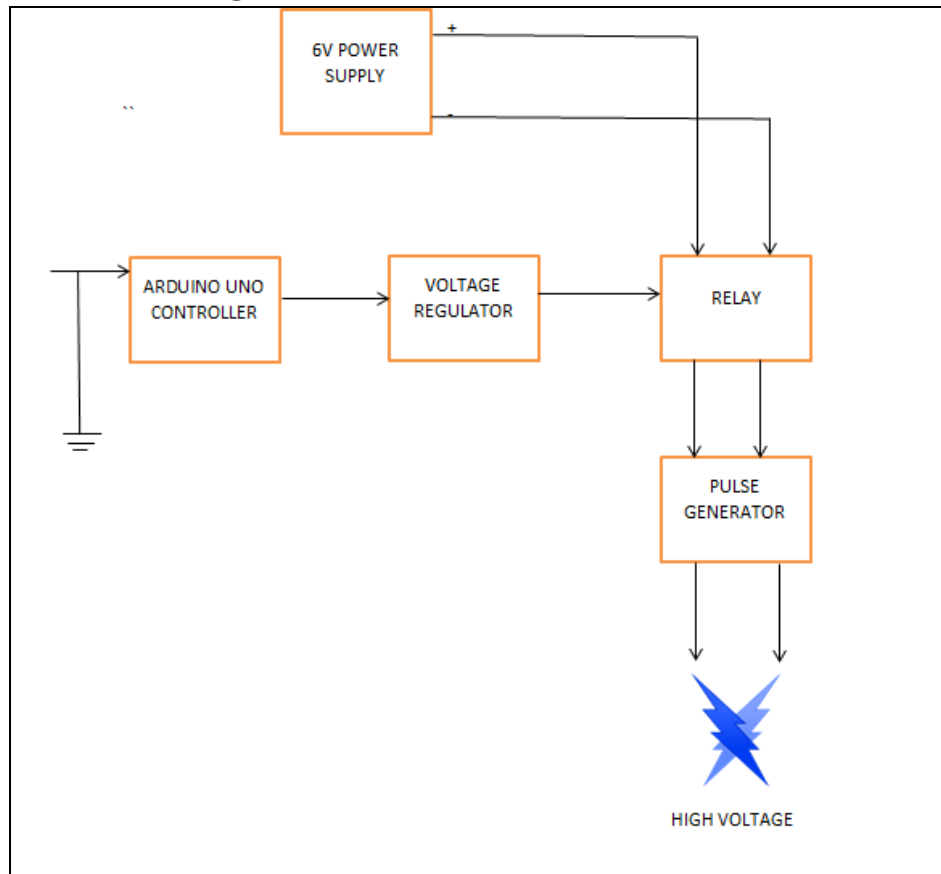


Figure 8 transistor

One of the most common uses for transistors in an electronic circuit is as simple switches. We also used a 2N2222 transistor in figure as a switch to help the Arduino UNO turn on and off the relays. In short, a transistor conducts current across the collector-emitter path only when a voltage is applied to the base. When no base voltage is present, the switch is off. When base voltage is present, the switch is on. Furthermore, it can reduce the noise from circuit that can disturb the Arduino. 470 ohm resistor was used before this transistor get signal from Arduino to decrease the voltage.

### 3.7 Controller design circuit



**Figure 9 Block diagram of square wave circuit**

The Figure 8 show how the Arduino communicate to pulse generator from the 5V battery source input to Arduino . Then from the controller pin no 13 to the voltage regulator that collect the signal to the relay. The relay must be powered by 6V from power source. Then from the relay it receive a signal from transistor to state ON or state OFF the relay.



### 3.9 TESTING OF CIRCUIT

We decided to implement and assemble all the circuit in the donut board which where the electronic component will be fixed and look tidy. Upon finished assemble the circuit, we proceed with the circuit testing to check whether the circuit is functioning or not. From the observation, formation of the spark at the pulse generator indicates that the electricity is produced in the circuit. At the same time, this test was performed to check the frequency and to vary the frequency for the electroporation process. After all we all run the system on the water first.

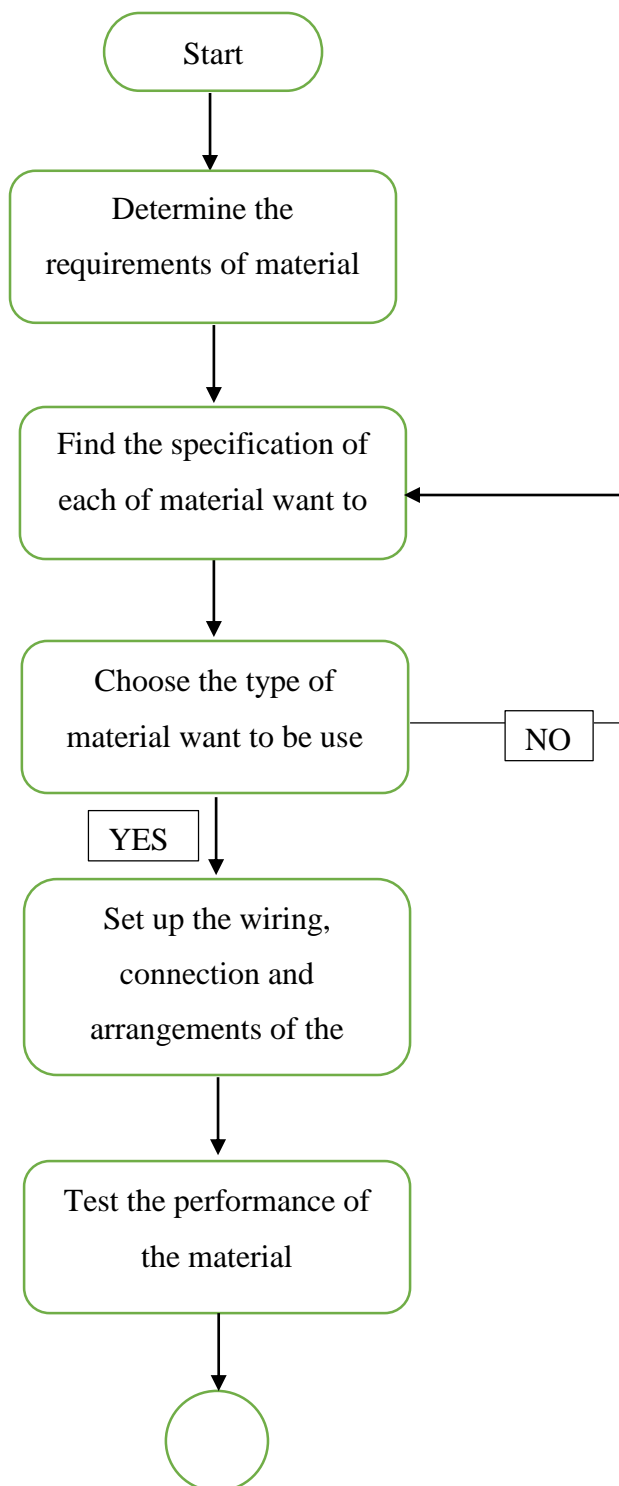


Figure 11: spark producing

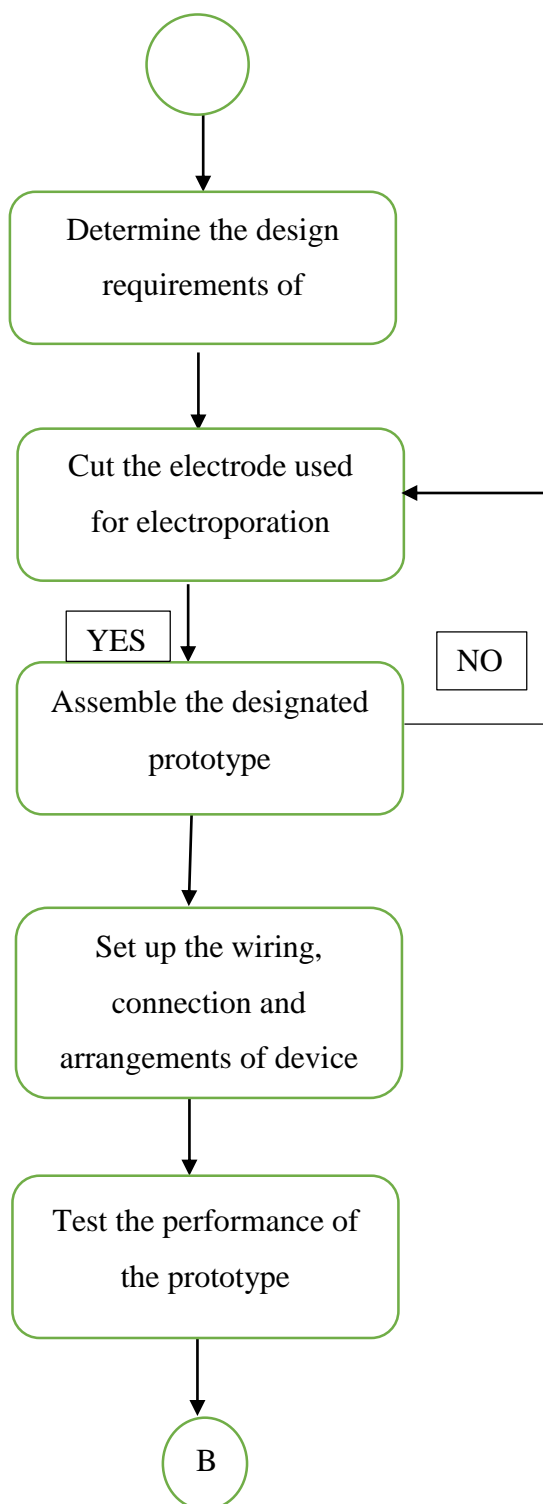
Water is one of the solution that being used in testing the efficiency of the electroporation process. Since water can be consider as a good conductivity, when we connect the electrode to the circuit, we can see there is reaction in the water indicate that the electricity.

### 3.10 FLOWCHART

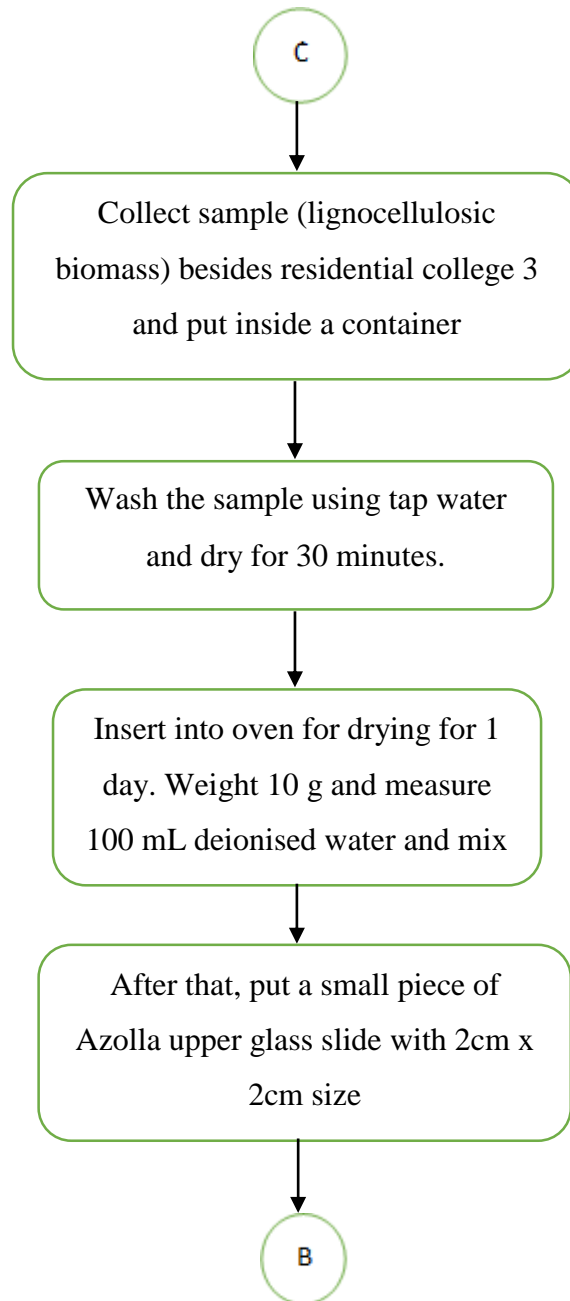
First phase : Determination of material for electroporation process



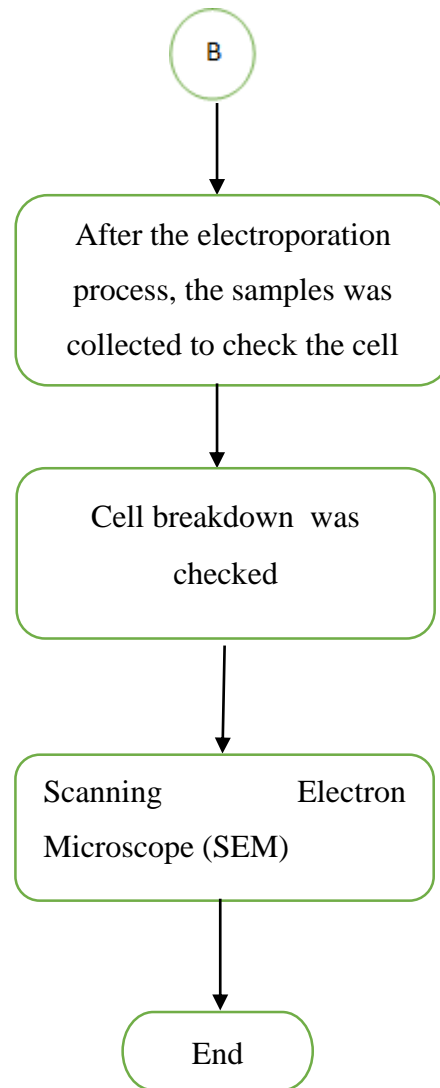
## Second phase : Determination of design for electroporation process



Third phase : how to test azolla



Fourth phase : Check for Cell Wall breakdown





## CHAPTER 4 RESULT AND DISCUSSION

### 4.1 Calculation of square wave output

Time (minutes)	Frequency (Hz)	Voltage (V)	Number of square wave (Pulse) $10^3$
0	490	5000	0
0.5	490	5000	2
1.0	490	5000	4
1.5	490	5000	6
2.0	490	5000	8
2.5	490	5000	10
3.0	490	5000	12
3.5	490	5000	14
4.0	490	5000	16
4.5	490	5000	18
5.0	490	5000	40

Table 1 show number of square wave during treatment process

## 4.2 CELLWALL BREAKDOWN

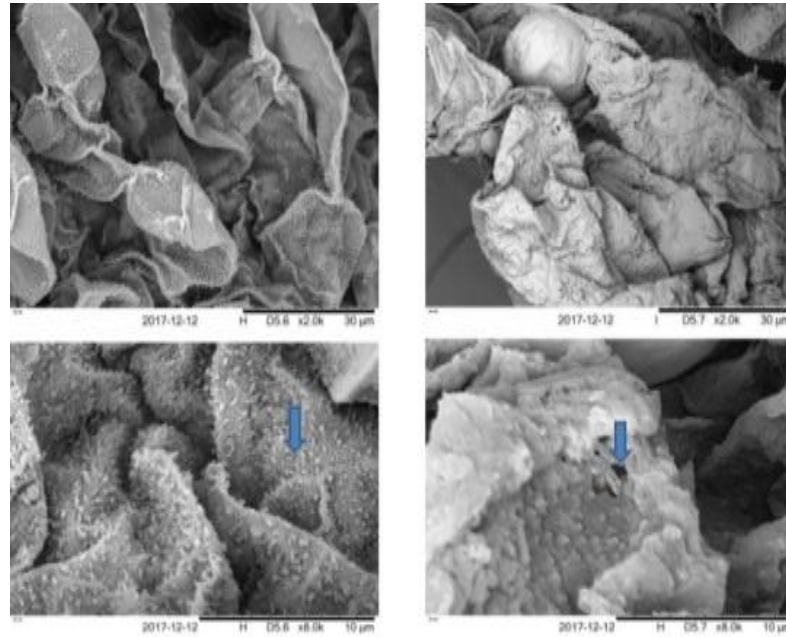


Figure 12 Effect of electroporation process

Fig.12 show the deformation structure of lignocellulosic biomass. The disruption bonding between cellulose, hemicellulose and lignin was happened because existed some hole when zooming using 8000 resolution of SEM EDX. It was clearly different surface of sample where before electroporation process, the surface was rough and look likes thorn. After electroporation process had done, the surface more smooth. This proven that after undergo electroporation process there were physical changes occur . We can compare the weight of lipid produce based on the distance between the electrodes and the treatment time. Due to the cell disruption effects during the electroporation process, intracellular lipids that come out to the surface of the treatment media can be extracted more efficiently and particularly with the longer treatment intensity and lower distance between the electrodes.

### 4.3 ETHICAL CONSIDERATION

We properly acknowledge other people's work and contribution in this project and from this research, It has been found that there is a need for power supply usage. As we know, power supply has a large dimension and difficult to bring everywhere. Furthermore, power supply also has a high number of energy consumed since it's draw a high electricity consumption to operate it. This has rise a problem of higher usage of energy consumption.

However, our project only use a battery as our source of electricity to run this electroporation process. It is more convenient rather than power supply since it does not require a plug or other sources of electricity. Before running this process, we had to setup all the materials such as cutting process to make an electrode sheets and construct a circuit diagram. All the process involved in this setup has been done without a supervision of expert and safety person. In this project, there are numerous process involving machine and electronic equipment.

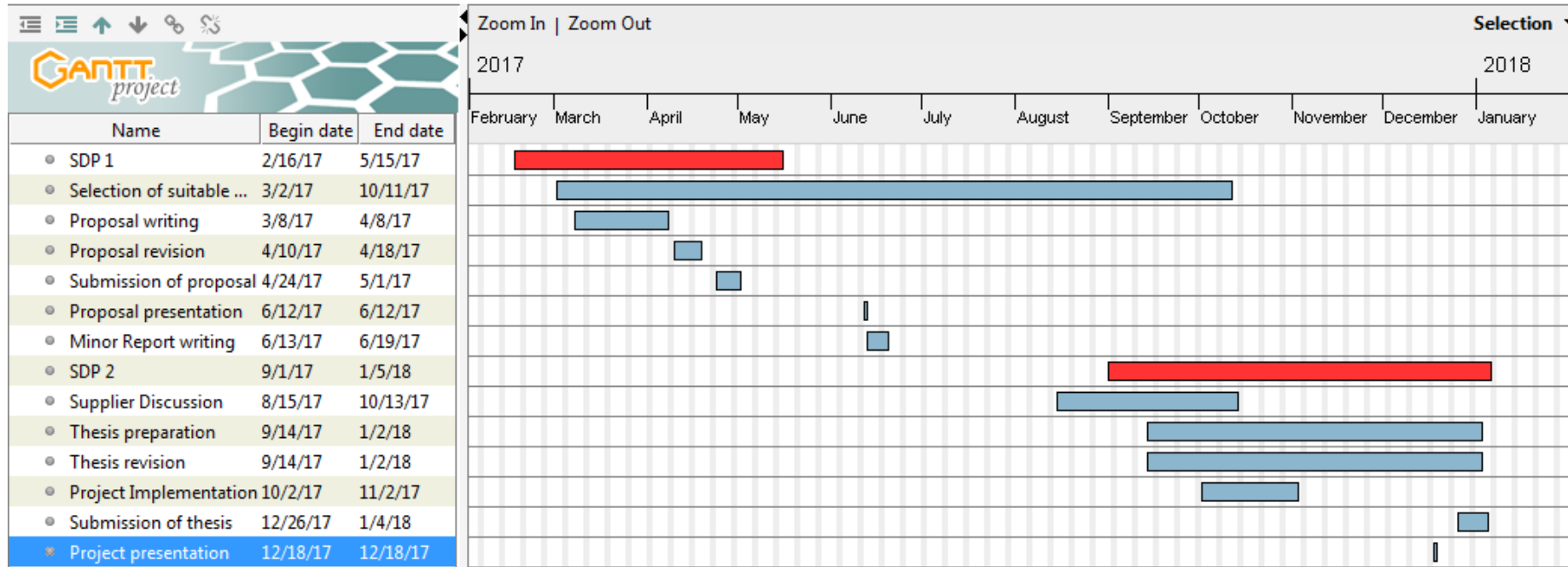
Thus, supervision from expert is a must to avoid unnecessary safety hazard during workshop. We have implement some safety precautions while doing this process such as wearing a safety boots, lab coat, gloves and mask to prevent any injuries from occurs. Furthermore, the design of the prototypes need to be sketched by using software such as Nx software to do the simulation and detail sketch up.

#### 4.4 COST ANALYSIS

Table 2 cost analysis

ITEM	COST/ UNIT	QUANTITY	COST (RM)
Pulse Generator	RM 21.40/unit	1 unit	RM 21.40
Single wire:- -black (5m) -red (5m) -green (5m)	RM 3/meter	10m	RM 30.00
Steel electrode	RM73.20	1 unit	RM 73.20
Resistor -220 ohm -0.22 ohm -470 ohm -27 k	RM 1/unit	5 unit	RM5.00
Transistor -PNP Type (2 unit) -NPN Type (1 unit)	RM 10/ unit	3 unit	RM 30.00
Capacitor	RM 1.20/unit	4 unit	RM4.80
Battery 6V 12 AH	RM 70.00	1 unit	RM70.00
1 way switch	RM 8.70	1 unit	RM8.70
Switch socket	RM 2.50	1 unit	RM2.50
PCB Stand	RM 1.00	5 unit	RM5.00
9V DC Connector	RM 2.40	2 unit	RM4.80
9V Battery	RM 8.00	4 unit	RM32.00
<b>TOTAL</b>			<b>RM287.40</b>

Table 3: Gantt chart



## CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

In this project, we have completed the designing and fabricating the treatment reactor for the electroporation process with the guidance from our supervisor, technical staff from our faculty and also among our friends. After some research and briefly discussion have been made, the electroporation method was choosed because have many advantage by keeping the environment clean and energy efficient as it also used electricity in shorter time.

The purpose of this project is to fabricate the design for the electroporation processes and also to observe the breakdown of the biomass to glucose due to the high voltage. Therefore, we can obtain more biofuel in the fastest way rather than use other method like chemical treatment, ultrasound and steam hydrolysis that have some disadvantage to environment and high cost to invest.

Furthermore, we also can improve the biofuel gas collection in more efficient way by fabricate the best design for the electroporation process. After the electroporation, the results that we obtain will be test by using several analysis techniques that are hydrolysis, FESEM and also the sugar analysis. In future, further studies are needed to optimize the electroporation process so that we can commercialize the application for more benefits. In addition, to expand this project, we can increase the voltage up to 15kV so that we can employ the voltage only in milliseconds while reduce the treatment time.

These techniques are used to determine the sugars and biogas produced from the electroporation process. We also will compare the sugars and biogas produced by using electroporation and without using electroporation uses these techniques. Our hypothesis is by using the electroporation, the sugars and biofuel produced will be more.

The potential of biofuels as one of energy source cannot be overlooked from the result of the present research because they can contribute considerably to supply energy for future. Moreover, since this the ground research, if we properly harness it, there will be a lot of improvement and development that can be made.

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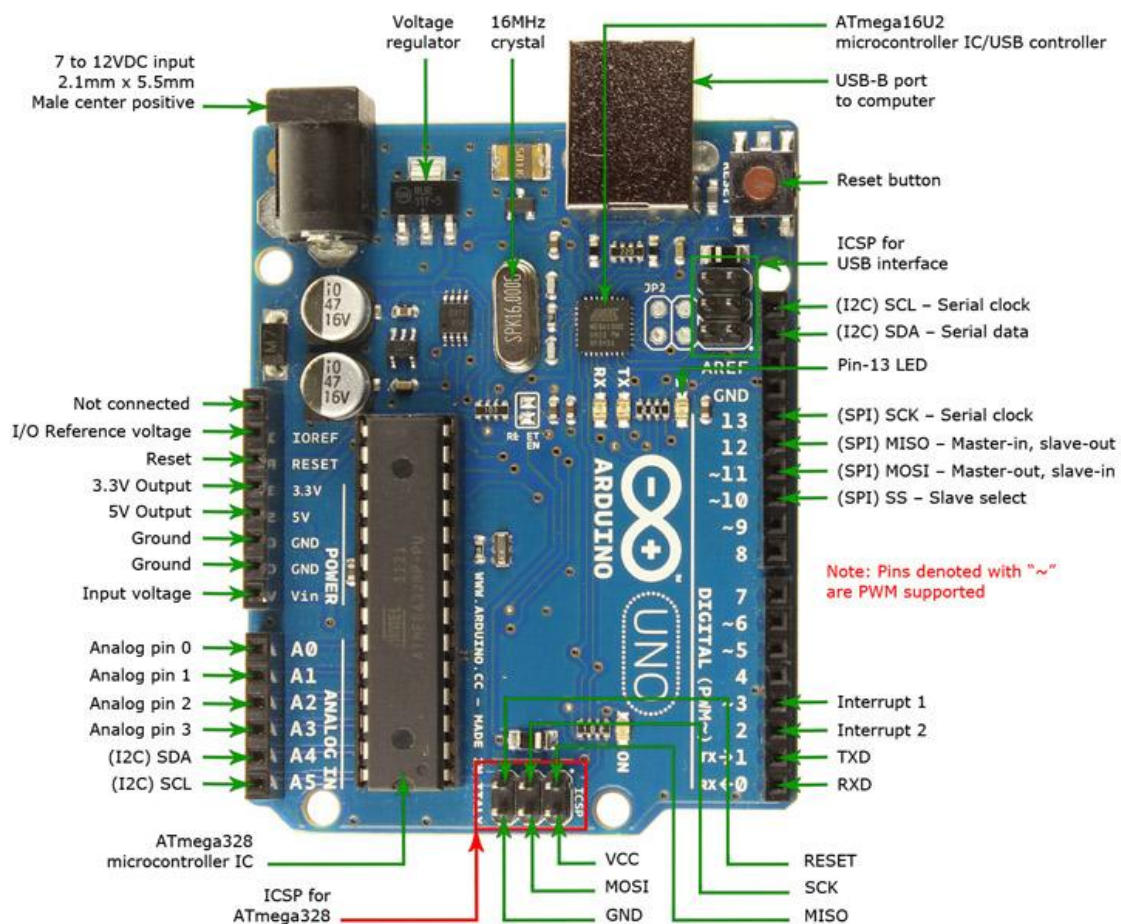
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## APPENDIXS

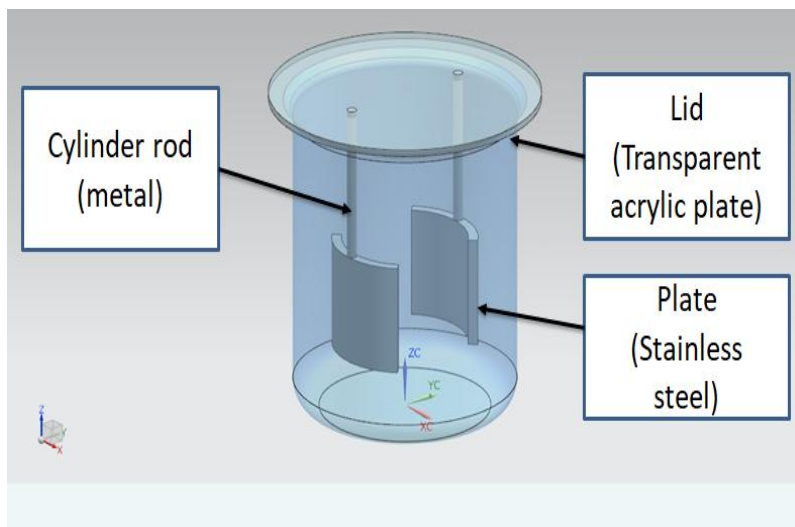
### APPENDIX A

#### Arduino Uno Pin out diagram



## APPENDIX B

## Electroporation device



(a)



(b)

Figure 13 Design (a) and fabrication (b) of electroporation device

## APPENDIX C

## Circuit fabrication



Figure 14 electroporation project

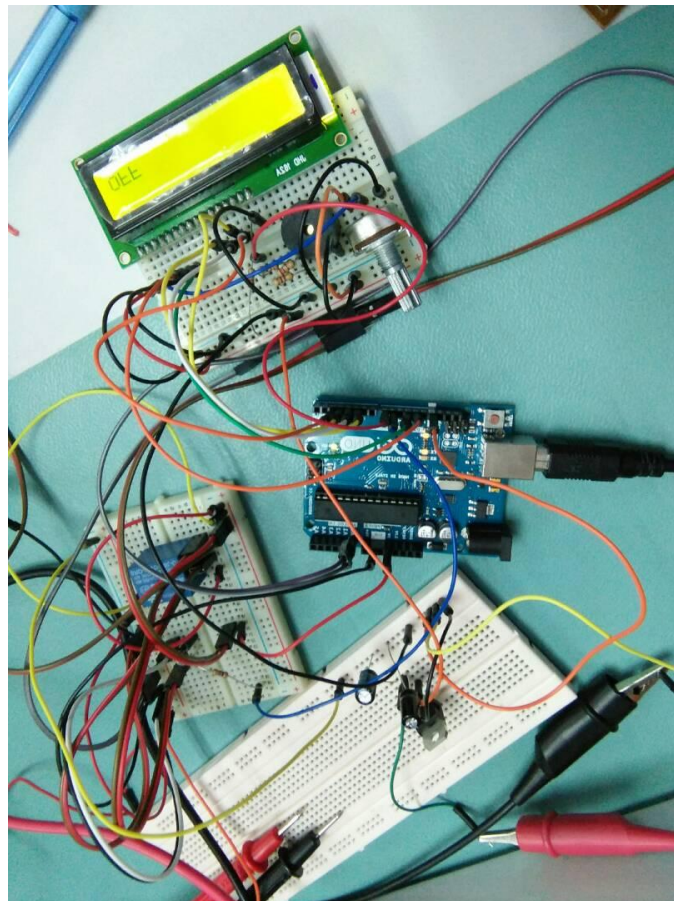


Figure 15 circuit development