ELECTROPORATION OF LIGNOCELLULOSIC BIOMASS

AARON KONG CHAK HO

Bachelor of Engineering Technology (Electrical) With Honors

UNIVERSITI MALAYSIA PAHANG

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Faculty of Engineering Technology
UNIVERSITI MALAYSIA PAHANG

ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance and the help of several individuals who contributed and extended their valuable assistance in the preparation and the completion of this study. Foremost, I would like to take this opportunity to express my sincere and deepest gratitude to my supervisor, Assoc. Prof. DR Che Ku Mohammad Faizal for the unlimited support, patience, motivation, guidance and knowledge for completing my thesis. Throughout the process of doing this senior design project (SDP), I have gained a lot of knowledge, skills and expertise such as time management skill, work together with project members inside the workshop, communication skill and so on. It is really an honour for me to work under his supervision as he has offered me his precious time and efforts in completing this SDP.

Apart from my supervisor, my sincere thanks to Professor Dato' Dr. Zularisam Ab Wahid, Dean of the Faculty of Engineering Technology, for providing me with all the necessary facilities and financial budget to complete the whole senior design project. My sincere thanks also go to the rest of the staffs from Faculty of Engineering Technology (FTEK) in University Malaysia Pahang (UMP), all the lecturers and JP's for helping and guiding me in the workshop for the circuit designs, product building, experimental analysis and data collection. Their kindness and patience offering helps are very much appreciated.

Throughout this whole project, I established good teamwork with my groupmates, Noor Atikah Binti Buang and Nor Nadhirah Binti Abdullah, and I would also like to take this opportunity to thank them for the great teamwork in order to finish this project as a whole.

Last but not least, I would like to express my utmost, sincere gratitude to both my parents and family members for all those motivations and encouragements throughout my studies, especially for the completion of senior design project. It's been a wonderful learning experience while working on this project.

ABSTRACT

Electroporation (EP) is versatility, rapid and high efficiency pretreatment technique using high voltage toward biomass feedstock. It is effectively and applicability to nearly all cell and species types. At the same time, Lignocellulosic biomass (*Pistia Stratiotes*) is the most abundant renewable bioresources on earth. The cellulose and hemicellulose contained in lignocellulosic biomass can be suitable substrate for bioethanol production. However, electroporation pretreatment technique still not widely use in this world. Therefore, this project was designing and fabricating an electric circuit and reactor to conduct electroporation treatment on lignocellulosic biomass (*Pistia*) in order to promote electroporation pretreatment technique. The performance of the circuit over the cell structure changes of the *pistia* will then test by doing electroporation treatment. In this project, Bluetooth HC-05 module was use as a region to communicate mobile phone with Arduino to control pulse width modulation (PWM) to produce high voltage square wave pulse. The high output voltage was produced by a DC step-up pulse generator which draw input source from 6V battery and controlled by SPDT relay for switching ON/OFF purpose. To prioritize the safety of user, LCD, buzzer and LEDs added to give signal and warning to user when the electric circuit is working. Bluetooth also one of the safety feature because user can control circuit by mobile phone and do not have to contact with the circuit to reduce the chance for getting shock. The reactor contains the container and electrodes with use to put the biomass sample and conduct treatment. Acrylic is chosen as the material to fabricate the container due to its transparency, electric and thermal insulated. Aluminum sheet was cut into small size to use as electrodes because it has high conductivity, not easy to get rust and low cost. Electroporation treatment is carried out to validate the electric circuit and reactor. The leaves of *pistia stratiotes* was dry in oven and then mashed into small particle to mix in water for treatment purpose. Sample was collected before treatment and during treatment from the mixture and then the sample cell structure analyzed under scale electron microscope (SEM). As a result, very obvious crack can be observed on the sample cell structure that undergo electroporation treatment compare to the sample cell before treatment. From the result, the cell structure of *pistia* was successfully disrupted, cellulose and hemicellulose can be extracted from it.

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LIST OF SYMBOLS

V	Volt
S	Second
min	Minute
SEM	Scale Electron Microscope
EP	Electroporation
IRE	Irreversible Electroporation
DNA	Deoxyribonucleic acid
kV	Kilo Volt
Ω	Ohm
Hz	Hertz
ms	Milli second

LIST OF ABBREVIATIONS

IDE	Arduino Software
I/O	Input / Output
SPDT	Single Pole Double Throw
DC	Direct Current
SPP	Serial Port Protocol
PWM	Pulse Width Modulation

CHAPTER 1

INTRODUCTION

In this chapter, the background and the problem statement are determined. Objectives and scope of study are identified to achieve the project purpose and overcome the problem statement.

1.1 BACKGROUD OF STUDY

Electroporation was initially developed for the introduction of DNA into cells which grow in suspension and was performed in a cuvette with two flat electrodes on opposite sides. Different configurations were subsequently developed for the electroporation of adherent cells in situ, while the cells were growing on nonconductive surfaces or a gold-coated, conductive support. We developed an assembly where the cells grow and are electroporated on optically transparent, electrically conductive indium-tin oxide (ITO). This material promotes excellent cell adhesion and growth, is inert and durable, and does not display spontaneous fluorescence, making the examination of the electroporated cells by fluorescence microscopy possible. The molecules to be electroporated are added to the cells and introduced through an electrical pulse delivered by an electrode placed on top of the cells.

Electroporation or electropermeabilization is a transformation technique that uses induction of macromolecular uptake by exposing cell walls to high-intensity electrical field pulses. The effectiveness of microalgal electroporation was first reported by Brown et al., 1991. Electroporation specifically disrupts lipid bilayers, leading to efficient molecular transport across the plasma membrane. Efficient electroporation-mediated transformation was achieved in both wild-type and cell wall–deficient Chlamydomonas cells (Brown et al., 1991). The transformation efficiency of electroporation is two orders

of magnitude higher than the glass beads method, and only requires relatively simple equipment. Important parameters affecting the effectiveness of electroporation include field strength, pulse length, medium composition, temperature, and membrane characteristics, as well as the concentration of DNA (Wang et al., 2007).

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. Irreversible electroporation (IRE or NTIRE for non-thermal irreversible electroporation) is a soft tissue ablation technique using ultra short but strong electrical fields to create permanent and hence lethal nanopores in the cell membrane, to disrupt the cellular homeostasis. The resulting cell death results from apoptosis and not necrosis as in all other thermal or radiation based ablation techniques.

1.2 PROBLEM STATEMENT

There are a lot of pretreatment methods on biomass feedstocks had already investigated. The alternative pretreatment of biomass feedstocks include ultrasound, microwave, extrusion, etc. Lignocellulosic biomass is one of the complicated pretreatment as the main obstacle for commercial use. Lignocellulosic feedstock materials are the most abundant renewable bioresource material available on earth. We are trying to make this pretreatment to be success and can be used more widely in biomass pretreatment so we are doing electroporation of lignocellulosic of biomass. We decided to use electroporation (EP) as the pretreatment to lignocellulosic biomass because electroporation is versatility. It is effectively and applicability to nearly all cell and species types. EP is easy, rapid and high efficiency. It able to transfect a large number of cells within shorter time when higher voltage is given. We are using Irreversible Electroporation (IRE) as the method to conduct the treatment on lignocellulosic of water lettuce (*Pistia Stratiotes*). IRE can fully damage the structure of the cell and cause the cell totally disrupted by high voltage pulse and only partially of the membrane can be repaired. Bioethanol can be produced by additional steps apply to the liquid that take from the structure of *Pistia* after EP treatment. The electroporation machine which available on market is not so convenient to carry and need to control by human themselves with contacting with the equipment, it may unsafe for user. From here, we are

trying to solve this problem and come out the objective of fabricate a Bluetooth based electric circuit and reactor to conduct electroporation. The portable smaller size of the device might easy for user to carry. As required, high voltage square wave pulse must be created for electroporation pretreatment technique.

Bioethanol is a renewable resource and also the most useful solution to all the issues related to environment and energy crisis. Bioethanol is biodegradable and much less toxic than fossil fuels. Air quality can be improved by using bioethanol in older engines by reduce the amount of carbon monoxide produced by the vehicle. To promote Bioethanol production and its uses, we reduce the production cost by using cheap substrate which is lignocellulosic *Pistia* and also to obtain the suitable microorganisms which provide sufficient fermentation yield. *Pistia* consisting 27.55% of cellulose and 29.71% of hemicellulose can be suitable substrate for bioethanol production by providing pre-treatment to *Pistia* (Sivasankari. B, David Ravindran. A, 2016).

Lignocellulosic of *Pistia* is the most abundant resources, low cost pre-treatment skill and less harmful to environment, while electroporation is the most suitable pre-treatment skill to lignocellulosic biomass. So we suggest that electroporation of lignocellulosic biomass can be one of the most effective and useful skill to produce bioethanol and can be used widely in the future.

1.3 OBJECTIVE

1. To design a Bluetooth based electric circuit to produce high voltage square wave pulse.

2. To fabricate a reactor to conduct electroporation.

3. To validate the performance of circuit over the structure changes of the lignocellulosic biomass (*Pistia Stratiotes*).

1.4 SCOPE OF STUDY

The application of electroporation (EP) is a new technology to disrupt or destroy any structure of biomass. Our project focused on the application of electroporation on the *Pistia Stratiotes* (Water Lettuce) cell structure disruption.

Electroporation is the increase of the permeability and conductivity of cell membrane due to an external applied of high pulse electric fields. When the strong electrical pulse was applied toward the cell the structures of the cell would be broke and rearranged to cause the permeabilization of the cell membrane to be increased. (U. Zimmermann, G. Pilwat, F. Friemann, 1974.) In our project, we are going to design and develop an electric circuit to conduct EP. The electric circuit of EP is designed to produce high voltage square wave pulse to disrupt the structure of the cell. We can use Arduino Board as the micro-controller to control the pulse width modulation. The electrical pulse must be in square waveform to give continuous pulse toward the cell. When the structure of the cell keeps hitting by the continuous pulse, the structure will be disrupted and break faster. Higher pulse voltage must be produced and applied to shorten the duration of the cell structure. The voltage pulse that apply to the cell structure should be higher than 1kV and this is the minimum pulse to conduct EP pretreatment. In our project we are doing voltage more than 10kV or maybe higher that must be under the safety condition. Theoretically, the higher the voltage pulse applied the shorter the duration to disrupt the structure of the cell.

In this project, we are fabricating a reactor to conduct the electroporation. Reactor is a container that contain of electrodes (positive and negative terminals) that connected to electrical circuit and can conduct the electric pulse field within the solution inside the container. The material to fabricate the electrodes is the most important factor to show better outcome. We must use the material that with higher reactivity and conductivity such as aluminium to speed up the reaction in the solution to get better outcome. The distance between electrodes should be consider. It can be one of the factor that affect the result. Another factor is the size and area of the electrodes, it will affect the amount of electromagnetic field that produce in between the electrodes thus affect the duration to disrupt the cell structure. The most significant size of electrodes and distance between electrodes can be observed and decide during the experiment.

After we done all our fabrication we will do some experiment to study and validate the performance of the circuit over the structure changes of the lignocellulosic biomass. We study the performance by changing the frequency or duty cycle of pulse width to see the performance and efficiency of the circuit. Then we will analyse the *Pistia* cell structure under microscope.

As this project will produce high amount of pulse voltage that maybe danger to user so we decide to use Bluetooth adapter module and connect into our electrical circuit. By adding Bluetooth module, user can control the circuit by using mobile software and can stand and observe the experiment at a safe distance. Incident can always happen at any time, we are trying to avoid this kind of situation happen during our experiment.

Our project as a small scale of prototype will be one of the greatest contribution to the world. It should be used wider in every field and should be studied deeper and detail to save our world as lignocellulosic of biomass is eco-friendly. We trust that lignocellulosic biomass can provide this world with more unexpected benefits. We must make good use of this great wealth of natural resources.

CHAPTER 2

LITERATURE REVIEW

In this chapter, the literature review on working principle of electroporation treatment technique is studied and the comparison on different pre-treatment technique with electroporation is stated.

2.1 INTRODUCTION

Lignocellulosic biomass was a composed of cellulose, hemicellulose and lignin. As biomass is an alternative to petroleum in production of biofuels and chemicals, it becomes valuable and recognized. Even today, cellulose consumption is threefold higher than that of steel and is equal to that of cereals (Das & Singh, 2004), but its current uses are mainly restricted to the materials sector (wood-based and paper). Lignocellulose (plant dry matter) such as wood, grass, agricultural, and forest residues, are potential resources for the production of bioethanol. Bioethanol is a suitable renewable or alternative energy source and also the potential solution of all the problems related with the environment and energy crisis. Cellulose cannot be digested by animal and human, thus developing lignocellulosic biomass as a feedstock becomes an ideal candidate for bioethanol production because it abundantly available and it also low in cost.

The current biochemical process of converting biomass to bioethanol typically consists of three main steps which are enzymatic hydrolysis, fermentation and pretreatment (Xu Z & Huang F, 2014). The enzymatic hydrolysis of lignocellulosic materials can be influenced not only by the effectiveness of the enzymes but also by the physical, chemical, and morphological characteristics of the lignocellulosic materials. Most factors that affect the enzymatic hydrolysis of lignocellulosic materials can be

primarily divided in two groups: enzyme-related and substrate-related factors (Alvira et al., 2010). Enzyme-related factors are mainly focused on improving the enzyme activity, including end product inhibition, thermal stability, synergism, and adsorption (Zhao et al., 2009). Substrate-related factors are mainly concentrated in improving the accessibility of enzymes to cellulose.

Fermentation is the biological process to convert the hexoses and pentoses into ethanol by a variety of microorganisms, such as bacteria, yeast, or fungi. Genetically engineered fungi that produce large volumes of cellulase, xylanase, and hemicellulose enzymes are under investigation. These could convert agricultural residues (e.g., corn stover, straw, and sugar cane bagasse) and energy crops (e.g., switch grass) into fermentable sugars. Additional research tried to find microorganisms which can effectively ferment both types of sugars into ethanol with Escherichia coli, Klebsiella oxytoca, and Zymomonas mobilis as promising candidates. (Qian Kang et al.)

For this process, pretreatment is probably the most crucial step since it has a large impact on the efficiency of the overall bioconversion. The aim of pretreatment is to disrupt recalcitrant structures of cellulosic biomass to make cellulose more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars. (Mosier et al. 2005) There are several pre-treatment process currently being studied that show potential in changing biomass to bioethanol such as organic solvent pretreatment, alkaline pretreatment technology, acid pretreatment and many more. But one of such technology is electroporation (EP).

Table 2.1 Pretreatment table of lignocellulosic biomass

PRE-TREATMENT TYPE	BIOMASS	COMPOSITION CHANGED	PRODUCT	REFERENCES
High Electric Field Pulse (Help)	Sugar Cane	Hemicellulose, Cellulose	Sugar	Rocha et al. (2011)
High Pressure Thermal	Chlorella Vulgaris	Hemicellulose, Cellulose	Bio Methane	Mende et al. (2014)
Alkaline	Corn Stover	Lignin	Ethanol	S. Donohoe et al. (2014)
Extrusion	Pine Wood Chips	Hemicellulose, Cellulose	Sugar	C et al. (2012)
Ozonolysis	Wheat Straw	Lignin	Sugar	Ben & Miron (1981)
Thermo-Mechanical	Soybean Hulls	Cellulose, Hemicellulose	95% Glucose	Yoo (2011)
Microwave	Switch Grass	Cellulose, Lignin	70-90% Sugar	Zhu et al. (2015a, b, 2016)
Ultrasound	Corn Starch	Cellulose, Hemicellulose	Bioethanol	Rehman et al. (2013)
Biological	Bamboo Culms	Lignin	90.9% Sugar	Suhara et al. (2012)
Electroporation (EP)	*Pistia Stratiotes*	*Cellulose, Hemicellulose*	*Bio Ethane*	*D. Mishima (2008)*

2.2 ELECTROPORATION (EP)

Electroporation phenomena can be traced back to the eighteenth century when red spots on human animal skin (Lichtenberg figures) were observed in the areas where electric fields were applied. Once the cause of this phenomenon was understood and control over the parameters that produce electric fields was achieved, a quick adoption of the use of pulsed electric fields to kill microbes was seen in the area of food and water sterilization (Rolong A. et al., 2018).

In terms of medical definition, electroporation is the application of an electric current to a living surface (as the skin or a cell membrane) in order to open pores or channels through which something (as a drug or DNA) may pass. Electroporation or electropermeabilization is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change a cell's function, or a piece of coding DNA (Jaquith, 2013). Electroporation was divided into two which are reversible electroporation (RE) and irreversible electroporation (IRE).

2.3 REVERSIBLE ELECTROPORATION

Reversible Electroporation (RE) is the temporary permeabilization of the cell membrane through the formation of nano-scale pores that are transient defects in the membrane. These pores are caused by short electrical pulses, typically on the order of a few to several hundred microseconds that are delivered by electroporation electrodes inserted around the treated tissue. Because the electricity applied is below the cells' threshold, it allows the cells to repair their phospholipid bilayer and continue their normal cell functions (Yair G. & Boris R., 2008). Reversible electroporation is typically done with treatments that involve getting a drug or gene (or other molecule that is not normally permeable to the cell membrane) into the cell. Not all tissue has the same electric field threshold; thus a careful calculations need to be made prior to a treatment to ensure safety and efficacy.

Electrochemotherapy is an important application of reversible electroporation in which the electrical permeabilization of the cell membrane is used to introduce into cells

drugs to which the cell membrane is otherwise impermeant. A 2002 MRI study of electrochemotherapy in nude mice with a laryngeal tumor has shown that without the addition of bleomycin, the reversible electroporation pulsed electric fields had no effect on T1W (A) images. The addition of bleomycin has produced changes in T1, at 24 hours after the treatment. With respect to T2, there was an effect of reversible electroporation, which disappeared at 48 hours (Mohammad Hjouj et al., 2012).

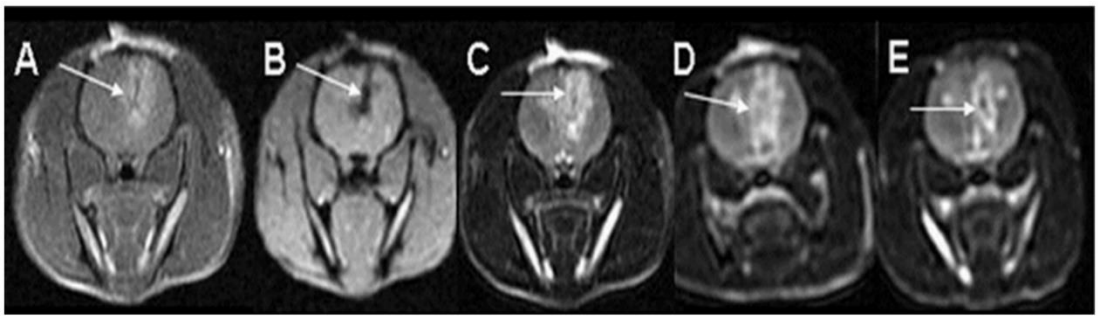


Figure 2.1 MRI sequences for depicting electroporation effects in the mice brain.

Source: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0042817

2.4 IRREVERSIBLE ELECTROPORATION

Irreversible Electroporation (IRE) is based on the principle of electroporation or electropermeabilization, in which electric pulses are used to create nano-scale defects in the cell membrane. These defects, termed “nanopores” or “conductive pores” permeate the cell membrane, permitting molecules to pass into targeted cells. Nanopore formation can be temporary (RE), as used in the fields of gene transfection and others. Different with the reversible electroporation, this method will cause the cell death due to the inability to maintain the homeostasis (IRE). This method of electroporation was used in the food industry for sterilization and more recently in medicine for tumour ablation. (Peter GK Wagstaff et al., 2016).

The presence of nanopores following the delivery of electrical pulses has been visualized using electron microscopy, showing the permanent and temporary nanopores (Lee EW et al., 2012). The pulse-induced disturbances of the cells as a whole were studied using fluorescence microscopy. This is because, the direct visualization of cell pores are difficult to follow over time. Thus, researchers have used indirect measures to quantify

the effect of electroporation. Changes such as in electrical conductivity or the uptake of dye following the admission of electrical pulses (Jiang CL et al., 2015). However, those changes does not definitely prove them to be the cause of IRE-induced cell death. But, using the animal research results have shown that IRE using the current clinically practiced treatment settings causes substantial increases in temperature in the targeted tissue. This secondary temperature development raises the question regarding the extent to the IRE effect that is caused by the temperature change versus time of the cell membrane to breakouts.

2.5 WORKING PRINCIPLE OF ELECTROPORATION

Electroporation is the application of controlled direct current (DC) electrical pulses using a pulse generator which are applied to living cells and tissues for a short duration of time in milliseconds. The pulse induces a transmembrane potential which causes the reversible breakdown of the cellular membrane. This action results in the permeation or “pore formation” of the cell membrane which allows small molecules. During this process the cellular uptake of the molecules continue until the pores close which can take milliseconds to minutes.

The phenomenon of electroporation can be described as a dramatic increase in membrane permeability caused by externally applied short and intense electric pulses. Various theoretical models were developed to describe electroporation, among which the transient aqueous pore model is the most widely accepted. According to this model, hydrophilic pores are formed in the lipid bilayer of a cell membrane when it is exposed to external electric pulses. In the cell membrane, hydrophobic pores are formed by spontaneous thermal fluctuations of membrane lipids. In a cell exposed to an external electric field, the presence of an induced transmembrane potential provides the free energy necessary for structural rearrangements of membrane phospholipids and thus enables hydrophilic pore formation (Neumann et al. 1989; Tsong 1991; Chang et al. 1992; Weaver and Chizmadzev 1996).

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, temperature, DNA concentrations and electroporation buffer.

2.6 APPLICATION OF ELECTROPORATION

From the last decade, the application of electroporation has been increasing rapidly. Nowadays, the electroporation technique can be applied in many way to deliver drugs, antibodies, oligonucleotides, proteins, RNA, DNA and plasmid in vivo for clinical, biotechnological and biomedical applications.

2.6.1 ELECTROPORATION IN BIOTECHNOLOGY

Electroporation both allows exogenous molecules to be introduced into cells and endogenous molecules to be extracted from within the cells, resulting in four general areas of biotechnological exploitation. In electrotransformation, exogenous DNA is introduced by means of reversible electroporation, the foreign genes are expressed in their new host cells and they are inherited upon cell division; this can turn the host microorganisms into ‘factories’ of biomolecules, adapt them to a new environment, or serve to study the role of individual genes. In electroporation-based inactivation, microorganisms are exposed to electric field pulses strong and long enough to inhibit their activity, including their division, growth, and synthesis of pathogenic substances. This method avoids contamination and is particularly promising in food preservation where radiation and chemicals must be avoided for the obvious reasons, while heating degrades both nutrients and taste, decreasing the value of food. In electroextraction, either microorganisms or multicellular tissues are electroporated to the extent required to release the biomolecules of interest; in some cases, it is also achievable with reversible electroporation and, in most cases, it is important to limit electroporation to levels that avoid rapid decomposition of the exposed cells and thus the formation of debris contaminating the extract. Finally, when used for facilitating water release from tissues, electroporation is useful in electroporative biomass drying, accelerating the drying process, allowing heating to be reduced or avoided, and often also reducing the energy requirements (Tadej Kotnik et al., 2015).

2.6.2 MEDICAL APPLICATIONS OF ELECTROPORATION

In vivo electroporation, first reported in 1987, makes it possible to render cell membranes temporarily permeable to substances that otherwise would not be able to effectively enter the cell interior. Micro- or millisecond pulses of electrical field strengths exceeding the natural cellular transmembrane potential difference of approximately 1V results in permeabilization ("poration") of cell membranes. This phenomenon opens up numerous applications in the medical field. Electroporative delivery of chemotherapeutic drugs into tumor cells has proven successful in clinical studies to treat malignant tumors and is nearing market Introduction in Europe. For gene therapy applications, delivery of DNA by electroporation into a variety of tissues has been shown to consistently result in a 100-1000-fold enhancement of gene expression. Other applications of electroporation discussed in this paper include intravascular delivery of drugs and genes with electroporation catheters, electroinsertion of molecules into membranes, intraocular delivery of drugs and genes, and transdermal drug delivery. The use of electroporation for drug and gene delivery in vivo is clearly gaining momentum, and new medical applications are emerging at an increasing rate (Dev, Rabussay, Widera, & Hofmann, 2000).

2.6.3 THE APPLICATION OF ELECTROPORATION TO TRANSFECT HEMATOPOIETIC CELLS AND TO DELIVER DRUGS AND VACCINES TRANSCUTANEOUSLY FOR CANCER TREATMENT

Electroporation and the associated phenomenon of electrofusion have been widely adapted as tools to a broad range of biomedical research and therapy. In this article, we summarize our adaptation of the electroporation and electrofusion technology in two fronts of cancer research and treatment. The first is genetic manipulation of hematopoietic cells for the purpose of cancer treatment. High efficiency transfection methods have been developed to transfect NK cells, peripheral blood stem cells, and bone marrow derived dendritic cells. Hybrids of tumor cells and bone marrow derived dendritic cells have been formed by electrofusion for the purpose of tumor vaccines. The second front is the use of transcutaneous electroporation to deliver anticancer drugs and vaccines across the skin. Methods to extend the upper molecular weight limit of transcutaneous

electroporation have been developed. The pro-photosensitizer drug, δ-amino levulinic acid, the anticancer drug methotrexate, and peptide vaccines designed for cancer prevention and immunotherapy have been delivered transcutaneously by electroporation. These studies hold promise for the treatment of cancers in human (Hui, 2002).

2.6.4 ELECTROPORATION FOR NANOMEDICINE

Nanoparticles have shown great promise in the development of efficient drug delivery systems, early diagnosis, and high-resolution imaging of hard-to-find diseases, such as early-stage malignant cancer and very rare circulating tumor cells, for pharmaceutical and medical applications. Recently, nanoparticles have been used as intracellular carriers of nano-drugs into targeted cells to release a slowly diffusing drug in the vicinity of the target disease. Several methods such as chemical reagent-based uptake, mechanical bombardment, direct injection, and electroporation have been developed to deliver nanoparticles into cells in a controlled manner. Unlike other methods, electroporation has continued to have great success with respect to the uptake efficiency, post-viability of cells, and high-throughput yield rate for numerous cell applications in association with nanoparticles. In this review, we present recent advances in the delivery of nanoparticles as intracellular carriers by electroporation (NICE) and highlight the salient features of NICE delivery at a multiscale level. We furthermore discuss the current challenges and future perspectives of NICE delivery for clinical applications (Lee, 2017).

CHAPTER 3

METHODOLOGY

This chapter included the materials and components use to construct the electric circuit. Flow chart of the system work and the circuit schematic diagram are show below.

3.1 MATERIALS AND COMPONENTS

3.1.1 PULSE WIDTH MODULATION (PWM) WITH ARDUINO MEGA BOARD

Pulse width modulation (PWM) is a method of Digital-Analog Conversion. Arduino can control analogue circuits with its digital outputs by using PWM technique. Normally in digital control it can only turn ON (5V) or OFF (0V) in the binary format. This turn on or off pattern can generate a square wave signal with constant frequency output. The fraction of the time of duty cycle can be varied from 0% until 100%. Same duty cycle at 50% will be use at different frequency of output to observe the result of the experiment.

Arduino is a microcontroller board that contains on-board power supply and USB port to communicate with PC. It able to read an input and turn it into an output. We can give instructions to the microcontroller on the board by making Arduino programming code using Arduino software (IDE) and upload to Arduino board (Figure 8). In this project we are using Arduino MEGA 2650 board. The board consists of 54 digital I/O pins (input / output) and 16 analogue pins which allowed more memory to store the code. We can obtain the power supplies to the circuit from the on-board 5V output power. It is really easy and convenient by using Arduino MEGA as microcontroller for our project.

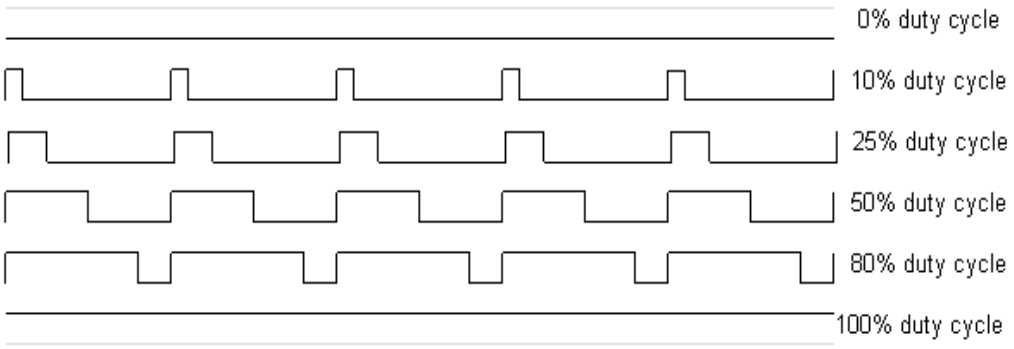


Figure 3.1 Duty cycle

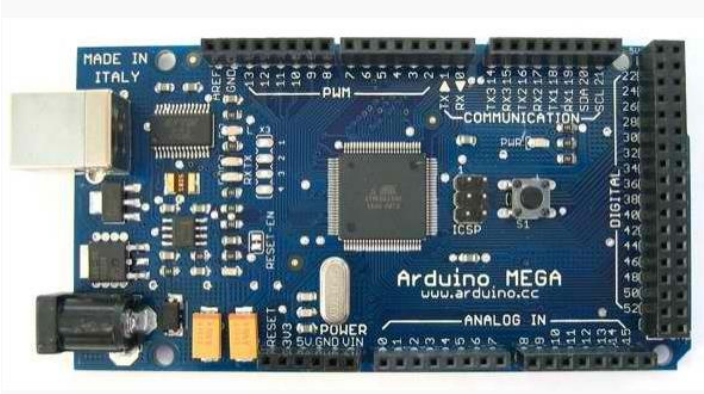


Figure 3.2 Arduino MEGA 2650 board

3.1.2 HC-05 BLUETOOTH MODULE

HC-05 Bluetooth module is an easy to use Bluetooth SPP (Serial Port Protocol) module which design for transparent wireless serial connection setup. It can be switching between master or slave mode which can neither receive nor transmit data. Bluetooth is use to communicate mobile phone with Arduino. It allowed mobile phone to connect with it and give instruction to Arduino. Different condition can be controlled by giving numbers command to control frequency of PWM to ON and OFF the output. Data from the Arduino that displayed on serial monitor also can be display on mobile phone synchronously. It also increases the safety of user when do the experiment by standing at the safe distance without contact with the circuit.

Figure 3.3 HC-05 Bluetooth module

3.1.3 OSCILLOSCOPE

Oscilloscope is an electronic test instrument to observe the change of an electrical signal over time. Some parameters such as amplitude, frequency, rise time, time interval can be get from the observed waveform. The oscilloscope is available in the laboratory. It is functioning to observe the square wave of the pulse width.

Figure 3.4 Oscilloscope

3.1.4 DC PULSE GENERATOR

Pulse generator act like a small step up transformer that can produce high output by small input voltage. This generator uses for Tesla coil principle to produce high voltage medium current. It also have small volume and high efficiency. 3.7V- 6V is needed as the supply source to operate the pulse generator. It can step up the DC input voltage to DC output voltage at the range from 200V up to 50kV. It is suitable to this project that need high output voltage. The high

output voltage use to disrupt the cell wall. Safety must always be prioritized since it is really high output voltage.

Figure 3.5 DC Pulse Generator

3.1.5 SPDT 6V RELAY

SPDT Relay stand for single pole, double throw electrically operated switch. Relay is an electrical device which use electromagnetic to operate as a switch. It was used to isolate two circuit electrically and connect them magnetically. Relay consists of two poles which are normally close (NC) and normally open (NO) allows one circuit to switch another one or act as “ON” and “OFF” like a switch when a power is given. Coil 1 and coil 2 are connected to 6V power input and 5V Arduino input respectively. COM is connected to 6V source to give power to NO pin when relay operate. The positive terminal of pulse generator is connected to NO and negative terminal to ground to complete the circuit operation.

Figure 3.6 SPDT 6V Relay

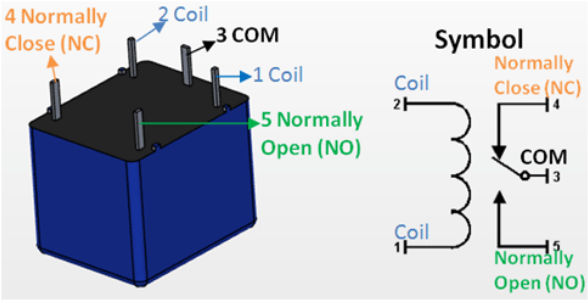


Figure 3.7 SPDT 6V Relay data sheet

3.1.6 RESISTOR

Resistor is a component that implements electrical resistances as a circuit element. It is mainly using to reduce the current flow within an electrical circuit. The resistance of a resistor is measured in ohms, Ω. We are using 220Ω resistor to reduce the power source flow in the components so it will not easily to burn. For example, reduce the brightness of LEDs. Figure 3.9 show the colour code that we can use to identify the resistance of the resistor.

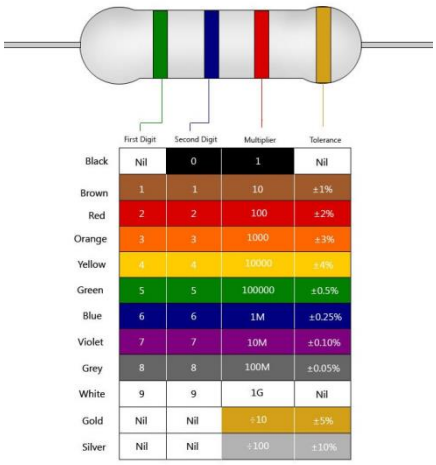


Figure 3.8 The colour code use to identify the resistance of a resistor

3.1.7 BATTERY

Battery act as a power source in a circuit. It is a device that covert chemical energy directly to electrical energy. The battery supplies the power to the electric device in this project. A portable product can be built by using battery source.

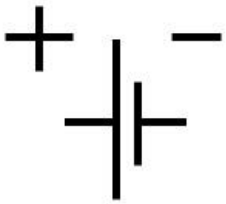


Figure 3.9 Symbol of DC battery voltage source

3.1.8 20X4 LIQUID-CRYSTAL DISPLAY (LCD)

20x4 LCD is an electronic module to display character. It can display 20 characters per line and there are 4 lines on LCD. We use LCD to display the reading which get from the circuit. User can understand the output by reading the display on the LCD.

Figure 3.100 20x4 Liquid-Crystal Display (LED)

3.1.9 TRANSISTOR 2N2222

Transistor 2N2222 is a common Negative-Positive-Negative (NPN) bipolar junction transistor (BJT) and function as low power amplifying or switching applications.

The transistor 2N2222 will only operate when power pass through Base Pin then allow Collector-Emitter (CE) current to flow.

Figure 3.111 Transistor 2N2222

3.1.10 LIGHT-EMITTING DIODES (LED)

LED is a semiconductor which emits light when electric current passes through it. We can also say that LED is a tiny light bulb. In this project, LEDs act as indicator light which indicate the user and alert the user when the circuit was operating.

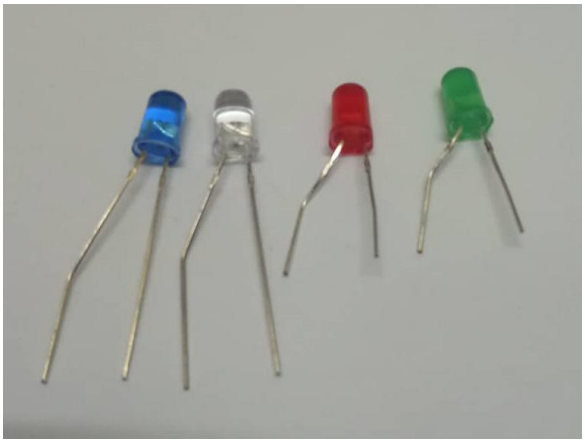


Figure 3.122 Light-Emitting Diodes (LED)

3.1.11 BUZZER

Buzzer is a small electronic device that giving signal by “beep” sound. In this project, buzzer is use to give alert to the user. It gives sound every time the Arduino give output. The circuit will operate after the signal sound from buzzer. It is one of the safety

feature in our project. User should beware with the high pulse voltage that produce by pulse generator when the treatment is started.

Figure 3.133 Buzzer

3.2 ELECTRICAL CIRCUIT DESIGN

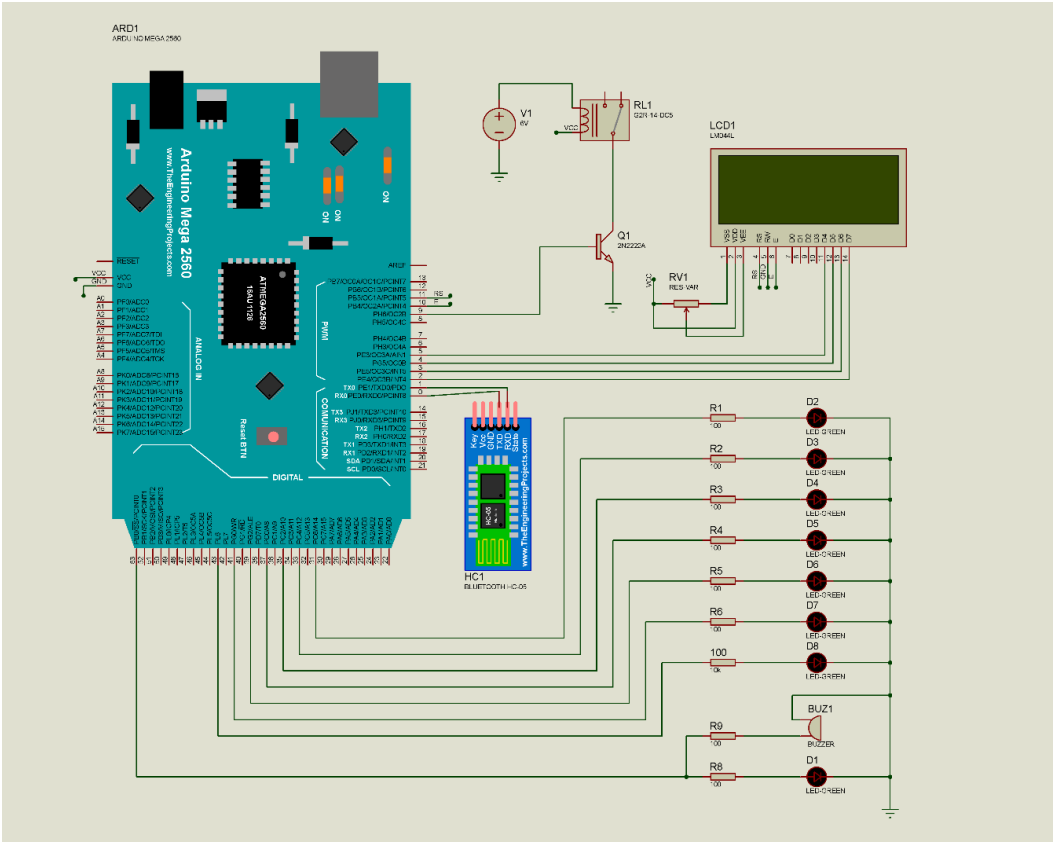
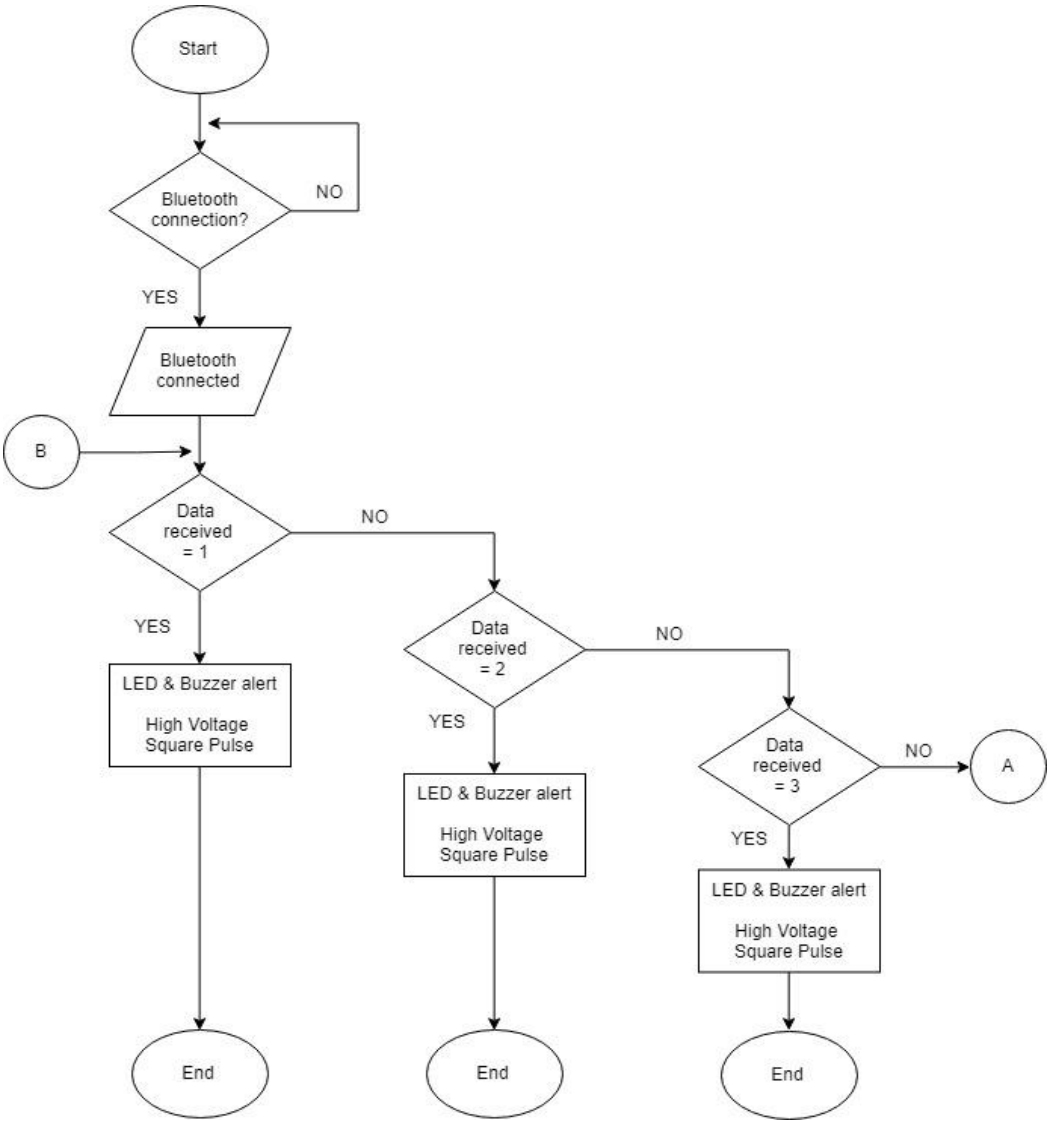


Figure 3.14 Electrical circuit schematic diagram

The electrical schematic circuit diagram was drew by using Proteus software. The hardware circuit will be connected according to the circuit design. Arduino is a microcontroller which can generate pulse width modulation (PWM) by making programming code using Arduino software (IDE) and upload to it. First of all, Bluetooth module HC-05 acts like a communication device that connect mobile phone with it to exchange data with the Arduino. When the Bluetooth is connected, the module HC-05 indicator light will blink slowly to notify user. Next, by using Arduino Bluetooth Controller software which downloaded from Android Play Store, user can give instruction to control the output frequency at the PWM output. Indicator lights (LEDs) are used to alert and give signal to user which step is happening. When instruction (data) is given to the Arduino, the PWM pin will start to send output to the transistor 2N2222 and then from the transistor to the relay. Both transistor and relay act like a switch which only operate when power pass through it to complete the whole circuit. When PWM is giving active HIGH (5V), transistor start to complete the circuit and relay start to operate and give output to the pulse generator. When PWM is LOW (0V), there is no output at the pulse generator. Continuous square wave created by continuously giving HIGH and LOW to the pulse generator. Output pulse voltage can be produce from 200V up to 50kV by the pulse generator. The Electroporation (EP) treatment to the lignocellulosic biomass can easily to disrupt by this continuous pulse and high voltage. The high pulse voltage will not cause human to dead but can hurt human body. A 20x4 LCD was use to display the reading from the circuit. When change is made on the instruction and it will display on LCD. It is functioning to make user to understand and know what happening on the circuit roughly.

3.3 SYSTEM FLOWCHART



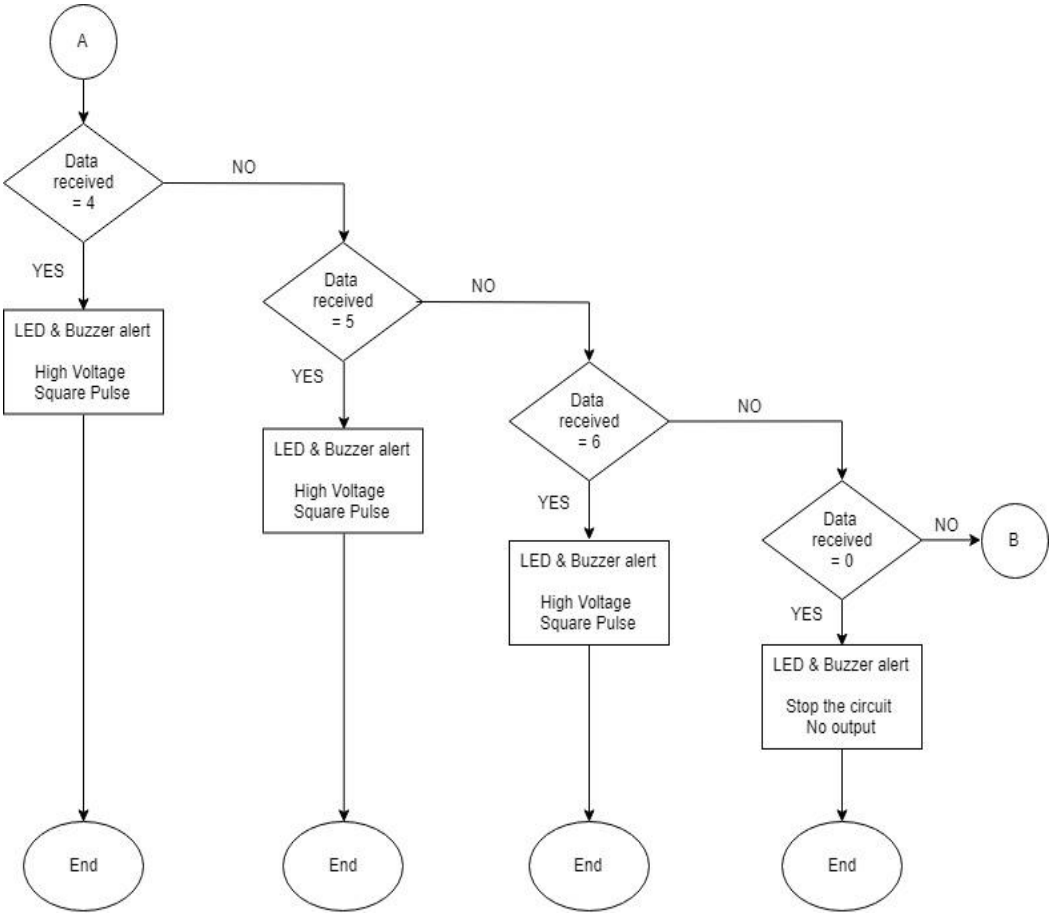


Figure 3.15 Flow of the system work

3.4 HARDWARE PROCESS

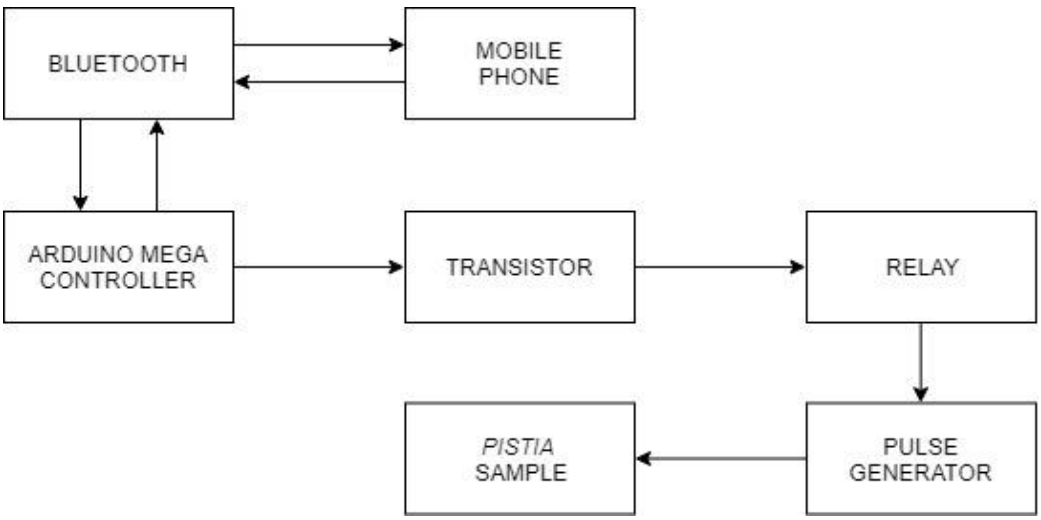


Figure 3.16 Block diagram of the hardware process

3.5 ARDUINO CODING (IDE)

```
// 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);      // lcd(rs, en, d4, d5, d6, d7)

// declare the following name and pin number
int PWM = 9;                    // PWM output pin at digital pin9
int PWM_out_level;
char data;
int LED1 = 31;
int LED2 = 33;
int LED3 = 35;
int LED4 = 37;
int LED5 = 39;
int LED6 = 41;
int LED7 = 43;                  // LEDS at digital pin31,33,35,37,39,41 and 43
int buzzer = 53;                // buzzer to arduino at digital pin 53
int T;

// Set up for the purpose
void setup() {
  Serial.begin(9600);           // set up to start serial display monitor in arduino
  lcd.begin(20, 4);             // set up the LCD's number of columns and rows
  pinMode(PWM, OUTPUT);         // set up PWM which at pin 9 as output
  pinMode(LED1,OUTPUT);         // set up the LEDS at the pin as output
  pinMode(LED2,OUTPUT);
  pinMode(LED3,OUTPUT);
  pinMode(LED4,OUTPUT);
  pinMode(LED5,OUTPUT);
```

```
  pinMode(LED6,OUTPUT);
  pinMode(LED7,OUTPUT);
  pinMode(buzzer, OUTPUT);          // set up the buzzer at pin 53 as output
  PWM_out_level = 255;              // set PWM at full speed which at 255
  }

// Loop all instruction in this section
void loop() {

// Bluetooth
  if(Serial.available() > 0)              // Send data only when you receive data
  { data = Serial.read();              // Read the incoming data & store into data

//when data was read then start to do the instruction

// Buzzer sound for 2 times everytime when receive data
  tone(buzzer, 500);                              // Send 500Hz sound signal
  delay(500);                                     // delay for 500milli second
  noTone(buzzer);                                 // Stop sound
  delay(500);                                     // and delay for 500ms
  tone(buzzer, 500);
  delay(500);
  noTone(buzzer);
  delay(500);
```

```
// LCD display when data receive
  lcd.setCursor (0,0);                  // set row 0 column 0 on LCD to display
  lcd.print("data = ");                 // display "data=" on LCD
  lcd.print(data);                      // read the reading from data
  lcd.setCursor (0,1);                  // set row 1 column 0 on LCD to display
  lcd.print("PWM  = ");                 // display "PWM=" on LCD row 2
  lcd.print(PWM_out_level);             // PWM full speed at 255
  }

  if(data == '0')       // Checks whether value of data is equal to 0 at serial.read()
   {
  digitalWrite( LED1,HIGH);             // if data = 0 , do the following
  digitalWrite( LED2,LOW);              // turn the LED1 ON, and the rest OFF
  digitalWrite( LED3,LOW);
  digitalWrite( LED4,LOW);
  digitalWrite( LED5,LOW);
  digitalWrite( LED6,LOW);
  digitalWrite( LED7,LOW);
  digitalWrite(PWM,LOW );
        // turn the pulse generator off by making the voltage LOW
  delay(500);                   // delay for 500milli second

  lcd.setCursor (0,2);              // set row 2 column 0 on LCD to display
  lcd.print("time = ");             // display "time=" on LCD
  lcd.print(T=0);                   // Read the reading from T
  lcd.print("ms     ");             // Display milli second (ms) after display time
  lcd.setCursor (0,3);              // set row 3 column 0 on LCD to display
  lcd.print ("THERE IS NO OUTPUT  ");
        // Display "THERE IS NO OUTPUT!" on LCD
    }
```

```
else if(data == '1')                // Checks whether value of data is equal to 1
 {
digitalWrite( LED1,LOW);       // if data = 1 , do the following
digitalWrite( LED2,HIGH);      // turn the LED2 ON, and the rest OFF
digitalWrite( LED3,LOW);
digitalWrite( LED4,LOW);
digitalWrite( LED5,LOW);
digitalWrite( LED6,LOW);
digitalWrite( LED7,LOW);
digitalWrite( PWM, HIGH);       // ON the PWM for 100ms to make 10HZ
delay(100);
digitalWrite(PWM,LOW );
    // Turn the pulse generator off by making the voltage LOW for 100ms
delay(100);
   // the ON and OFF time at the same value cause a constant 50% duty cycle

lcd.setCursor (0,2);
lcd.print("time = ");
lcd.print(T=100);
lcd.print("ms ");
lcd.setCursor (0,3);
lcd.print("BEWARE!HIGH OUTPUT! ");
                        // Display "BEWARE!HIGH OUTPUT!" on LCD
}

else if(data == '2')           // Checks whether value of data is equal to 2
  {
digitalWrite( LED1,LOW);       // if data = 2 , do the following
digitalWrite( LED2,LOW);       // turn the LED3 ON, and the rest OFF
digitalWrite( LED3,HIGH);
digitalWrite( LED4,LOW);
digitalWrite( LED5,LOW);
```

```
digitalWrite( LED6,LOW);
digitalWrite( LED7,LOW);
digitalWrite( PWM, HIGH);       // ON the PWM for 200ms to make 5HZ
delay(200);
digitalWrite(PWM,LOW );
        // Turn the pulse generator off by making the voltage LOW for 200ms
delay(200);
       // the ON and OFF time at the same value cause a constant 50% duty cycle

lcd.setCursor (0,2);
lcd.print("time = ");
lcd.print(T=200);
lcd.print("ms ");
lcd.setCursor (0,3);
lcd.print("BEWARE!HIGH OUTPUT! ");
}

else if(data == '3')                  // Checks whether value of data is equal to 3
  {
digitalWrite( LED1,LOW);        // if data = 3 , do the following
digitalWrite( LED2,LOW);        // turn the LED4 ON, and the rest OFF
digitalWrite( LED3,LOW);
digitalWrite( LED4,HIGH);
digitalWrite( LED5,LOW);
digitalWrite( LED6,LOW);
digitalWrite( LED7,LOW);
digitalWrite( PWM, HIGH);       // ON the PWM for 300ms to make 3.33HZ
delay(300);
digitalWrite(PWM,LOW );
// Turn the pulse generator off by making the voltage LOW for 200ms
 delay(300);
// the ON and OFF time at the same value cause a constant 50% duty cycle
```

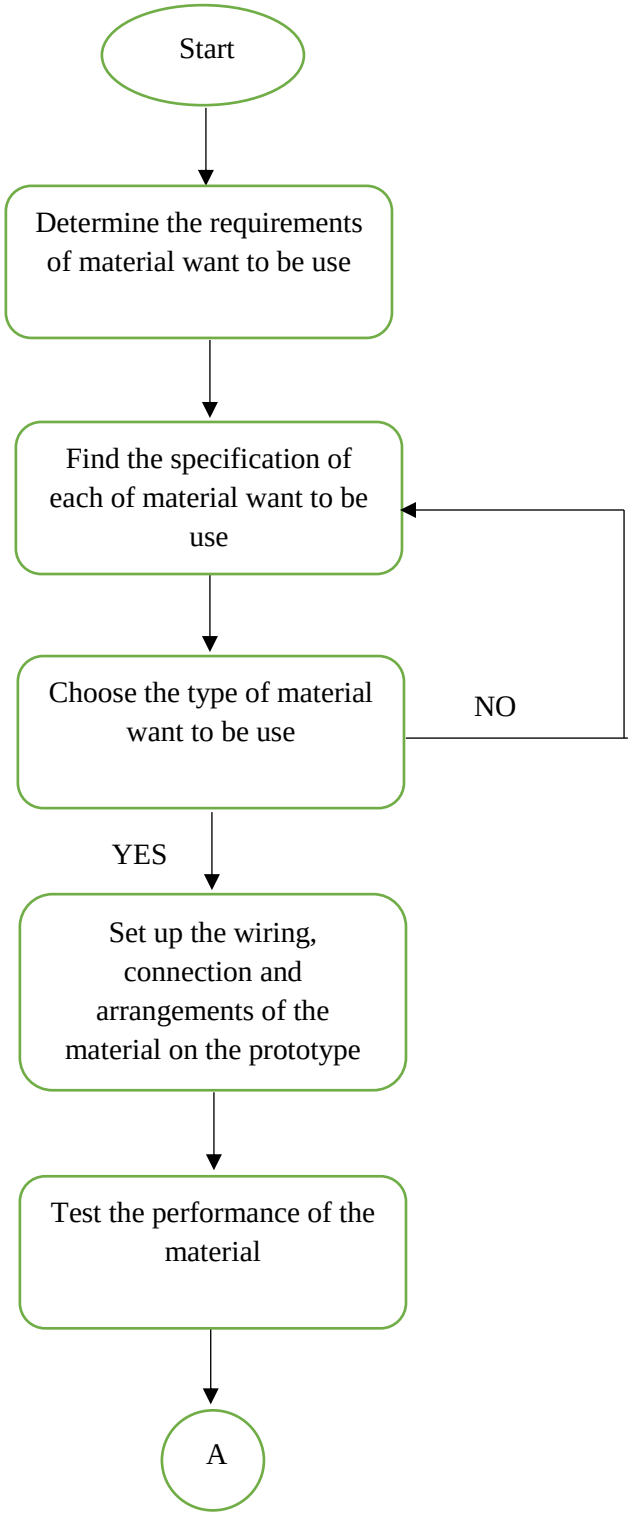
```
lcd.setCursor (0,2);
lcd.print("time = ");
lcd.print(T=300);
lcd.print("ms ");
lcd.setCursor (0,3);
lcd.print("BEWARE!HIGH OUTPUT! ");
}

else if(data == '4')          // Checks whether value of data is equal to 4
  {
digitalWrite( LED1,LOW);       // if data = 4 , do the following
digitalWrite( LED2,LOW);       // turn the LED5 ON, and the rest OFF
digitalWrite( LED3,LOW);
digitalWrite( LED4,LOW);
digitalWrite( LED5,HIGH);
digitalWrite( LED6,LOW);
digitalWrite( LED7,LOW);
digitalWrite( PWM, HIGH);      // ON the PWM for 400ms to make 2.5HZ
delay(400);
digitalWrite(PWM,LOW );
        // Turn the pulse generator off by making the voltage LOW for 400ms
delay(400);
         // the ON and OFF time at the same value cause a constant 50% duty cycle

lcd.setCursor (0,2);
lcd.print("time = ");
lcd.print(T=400);
lcd.print("ms ");
lcd.setCursor (0,3);
lcd.print("BEWARE!HIGH OUTPUT! ");
}
```

```
else if(data == '5')          // Checks whether value of data is equal to 5
  {
 digitalWrite( LED1,LOW);       // if data = 5 , do the following
 digitalWrite( LED2,LOW);       // turn the LED6 ON, and the rest OFF
 digitalWrite( LED3,LOW);
 digitalWrite( LED4,LOW);
 digitalWrite( LED5,LOW);
 digitalWrite( LED6,HIGH);
 digitalWrite( LED7,LOW);
 digitalWrite( PWM, HIGH);       // ON the PWM for 500ms to make 2HZ
 delay(500);
 digitalWrite(PWM,LOW );
        // Turn the pulse generator off by making the voltage LOW for 500ms
 delay(500);
        // the ON and OFF time at the same value cause a constant 50% duty cycle

 lcd.setCursor (0,2);
 lcd.print("time = ");
 lcd.print(T=500);
 lcd.print("ms ");
 lcd.setCursor (0,3);
 lcd.print("BEWARE!HIGH OUTPUT! ");
 }

 else if(data == '6')          // Checks whether value of data is equal to 6
  {
 digitalWrite( LED1,LOW);       // if data = 6 , do the following
 digitalWrite( LED2,LOW);       // turn the LED7 ON,and the rest OFF
 digitalWrite( LED3,LOW);
 digitalWrite( LED4,LOW);
 digitalWrite( LED5,LOW);
 digitalWrite( LED6,LOW);
```

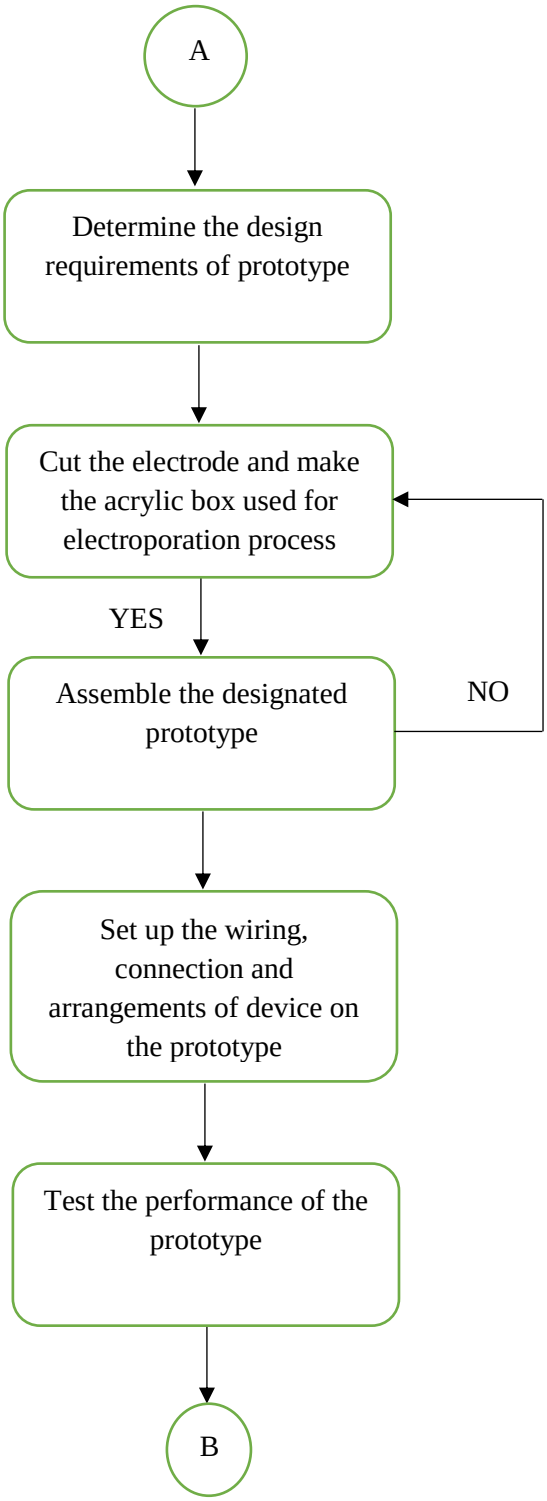
```
 digitalWrite( LED7,HIGH);
 digitalWrite( PWM, HIGH);       // ON the PWM for 600ms to make 1.67HZ
 delay(600);
 digitalWrite(PWM,LOW );
        // Turn the pulse generator off by making the voltage LOW for 600ms
 delay(600);
        // the ON and OFF time at the same value cause a constant 50% duty cycle

 lcd.setCursor (0,2);
 lcd.print("time = ");
 lcd.print(T=600);
 lcd.print("ms ");
 lcd.setCursor (0,3);
 lcd.print("BEWARE!HIGH OUTPUT! ");
 }

//serial monitor display
//Print Value inside data in Serial monitor
 Serial.print("\n");                  // Print word in the next line
 Serial.print("PWM  = ");             // Print "PWM=" on serial monitor
 Serial.print(PWM_out_level);        // Read the reading from PWM_out_level
 Serial.print("\n");                  // Print word in the next line
 Serial.print("TIME = ");             // Print "TIME="
 Serial.print(T);                    // read from T to display on time
 Serial.print("\n");                  // Print word in the next line
 Serial.print("data = ");             // Print "data = "
 Serial.println(data);               // Read the reading from data
}

//END
```

3.6 FLOWCHART OF ACTIVITIES

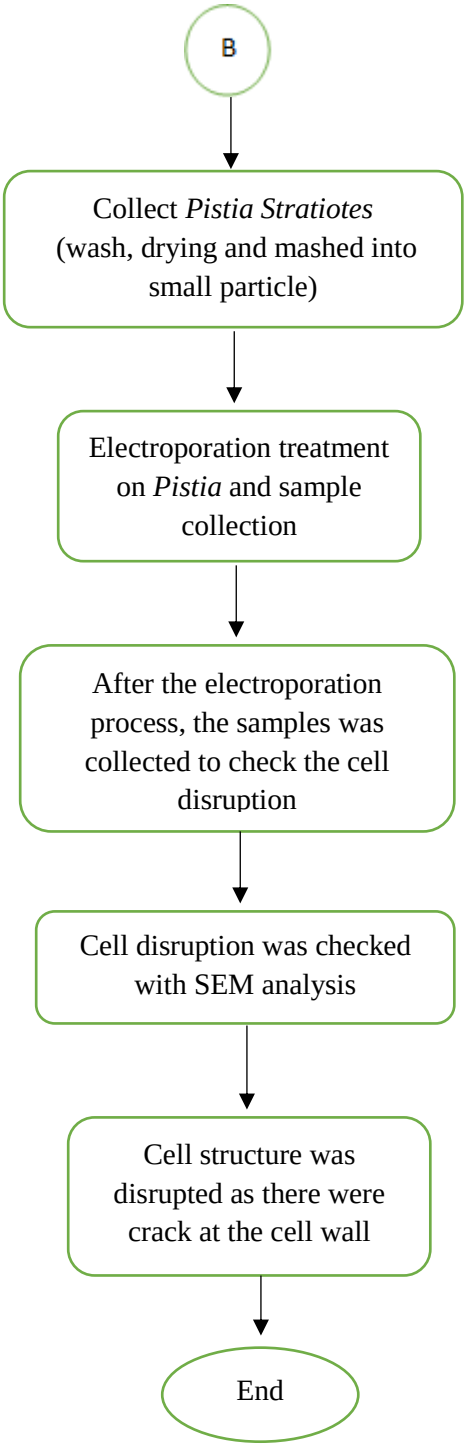
First stage: Determination of material for electroporation process



Phase 2: Determination of design for electroporation process



Phase 3: Validate the Performance of Circuit and Check for Cell Structure Disruption



CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, the results of the final product in this project and the changes of *pistia stratiotes* cell structures before and after treatment will be discussed.

4.1 PULSE SQUARE WAVE

By using oscilloscope, square wave was observed which produced by the pulse width modulation (PWM) from the Arduino. The output of the pulse generator was operated due to this square wave. When the square wave was HIGH (5V), the pulse generator gives high output voltage to the sample. While, the square wave was LOW (0V), the pulse generator stops giving output. The ON/OFF time or can say as HIGH/LOW time is around 1second per cycle at frequency of 10Hz. Table 4.1 show the square wave produced for 20 minutes. It means that the high output voltage pulse during electroporation had be done for this much. More pulse can be produced for longer treatment time.

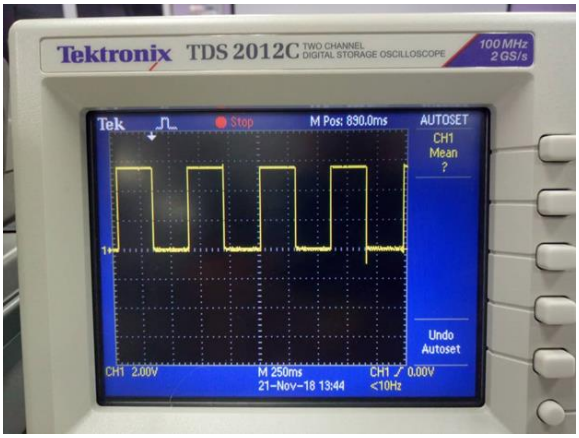


Figure 4.1 Square wave

Table 4.1 Number of square wave produced vary with time

Time (Minutes)	Frequency (Hz)	Voltage (kV)	Number of square wave (pulse)
0	10	30	0
5	10	30	1500
10	10	30	3000
15	10	30	4500
20	10	30	6000

4.2 ELECTRICAL FIELD

As the fabrication done, air bubbles were observed on the electrodes during circuit testing. The bubbles show that reaction was happening during electroporation (EP). During the electric shock, which when the output is giving HIGH, the cations and anions will be transfer toward the positive and negative terminal of the electrodes (Zoulias et al., 2002). When cations and anions are moving in the solvent, electromagnetic field will be created (Iida et al., 2007). The electrode which contain more bubbles was the cathode terminal, it is because the cathode terminal released the electron and received by anode terminal (Vanags et al., 2011b).

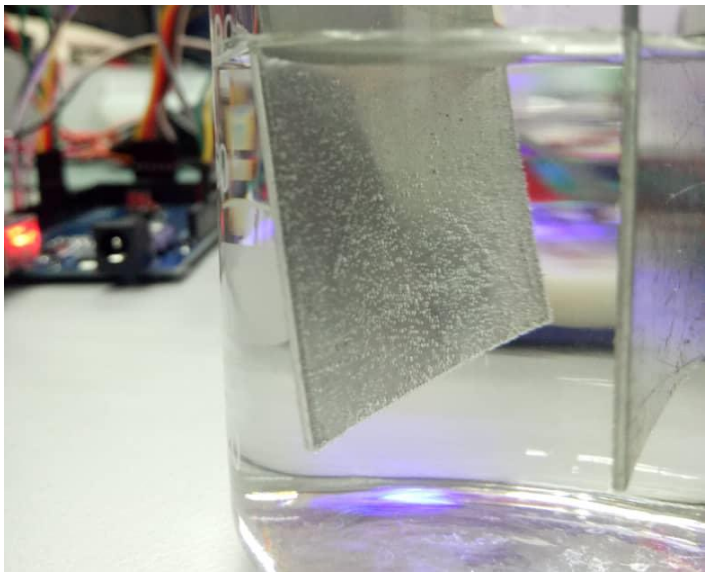


Figure 4.2 Bubbles on electrodes

4.3 DEVICE

The product includes of circuit and reactor. The circuit was first test on breadboard and then transfer to PCB donut board for better connection and tidy look. Crocodile clips was used to connect the pulse generator with the aluminium electrodes to conduct electroporation. Acrylic as a transparent material which allowed user to see the physical changes during electroporation was used to make the container. The top of the container can be open so user can put the sample into the container easily and close to cover from insect and dust to entering the mixture and effect the treatment process. The top cover of the container also easier the washing process.

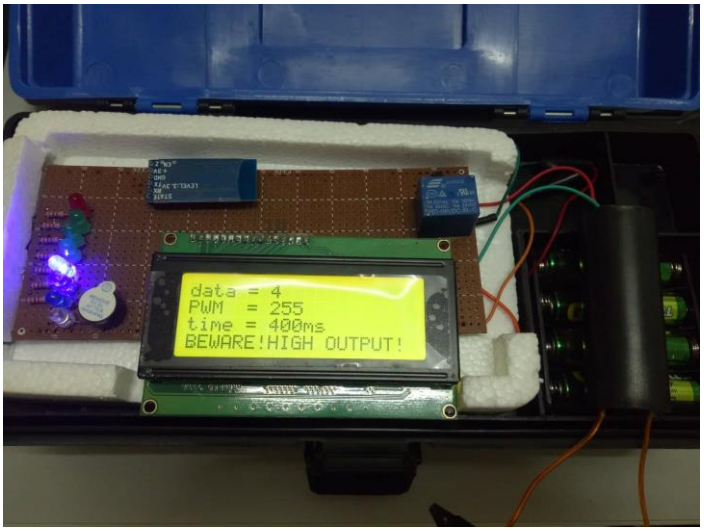


Figure 4.3 Product Hardware

Figure 4.4 Reactor

4.4 CHANGES OF *PISTIA STRATIOTES* CELL STRUCTURES

Pistia stratiotes was use as lignocellulosic biomass sample in this project. *Pistia* contains of lignin, cellulose and hemi-cellulose which suitable to this project title. The sample was first collected and dry in the oven under a specific temperature to remove the existing water in the cell. The dry sample then mashed into small particle or powder and then mix with water to conduct electroporation treatment. Sample was collected from the mixture before treatment and after treatment then observed by using Scale Electron Microscope (SEM-EDX) zooming skill to see the cell structures. The figures show the cell structures observation before and after treatment. Obvious breaking line can see on the cell wall after treatment compare to the cell wall before treatment.

Figure 4.5 Sample after EP treatment

From figure 4.5, brown colour solution can be observed due to the cellulose and hemicellulose had extracted from the *Pistia* cell. This is one of the proved that the electroporation treatment is successfully disrupt the cell structure of *pistia*.

Figure 4.6 show the look of *Pistia* cell structures before EP treatment. The cell structures are in good condition and no crack observe here.

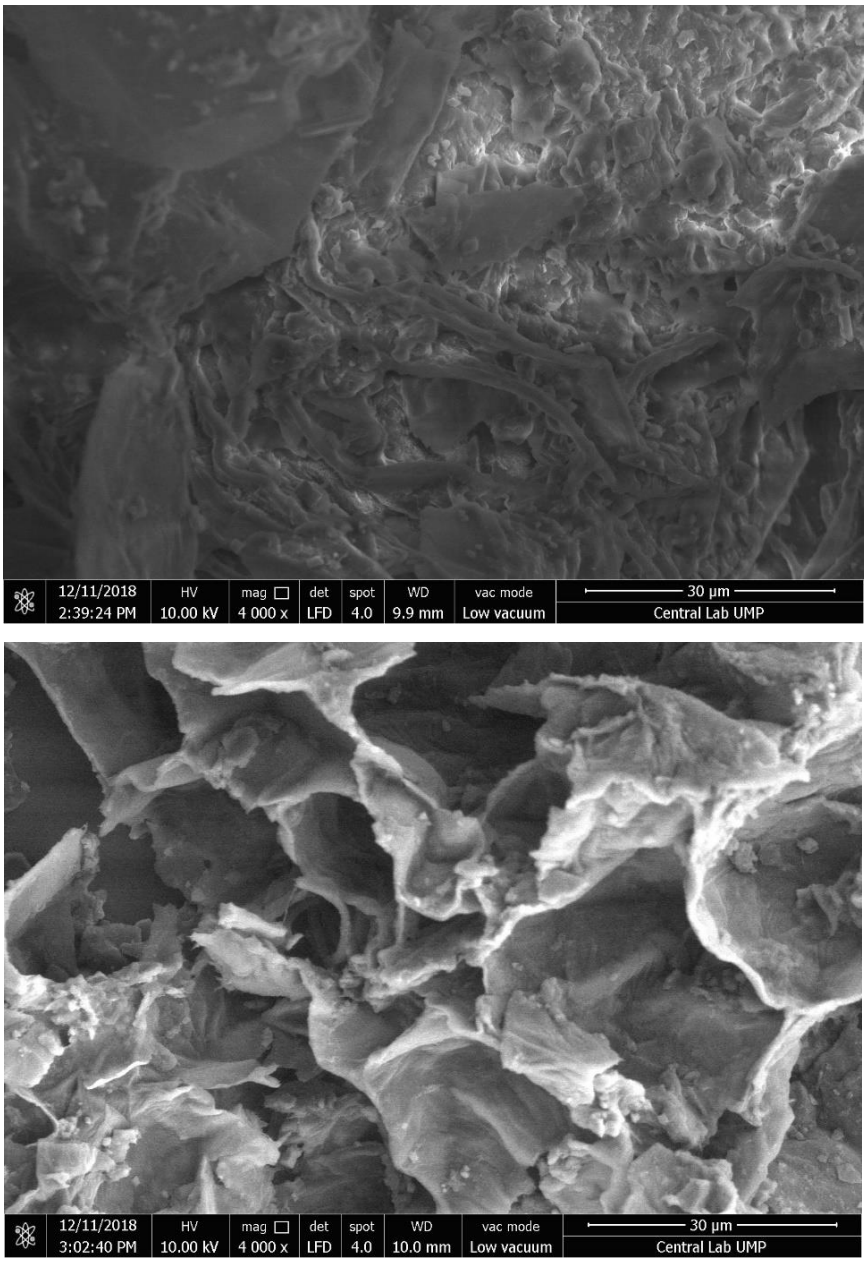


Figure 4.6 Cell structure before EP

Figure 4.7 show the cell structures after 10 minutes of electroporation treatment. From the figure, the lignin (outermost of the cell structure) no longer neatly and obvius crack can be observed on the cell wall.

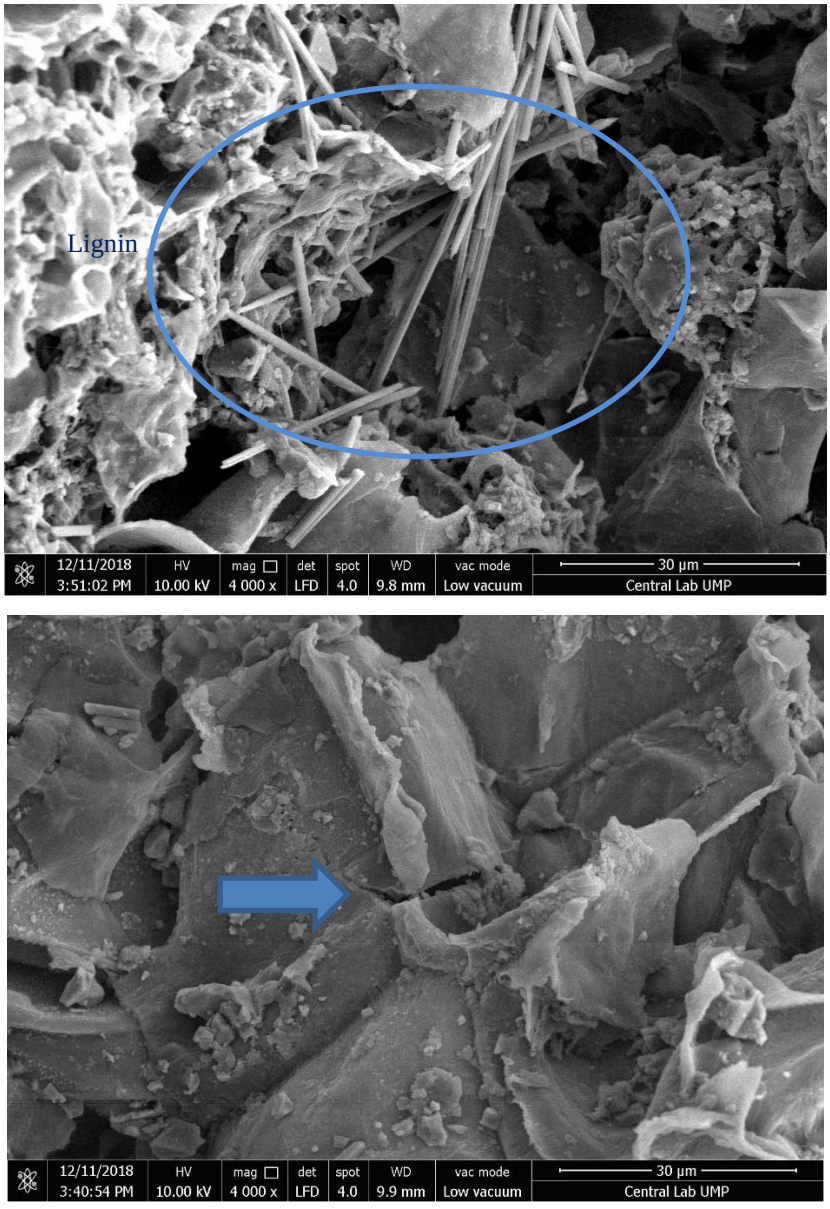


Figure 4.7 Cell structures after 10 minutes of EP treatment

Figure 4.8 show larger and longer crack on the cell structures of *pistia* compare to the cell structures at 10 minutes. The result shows the longer the treatment time, the effective the cell disrupted.

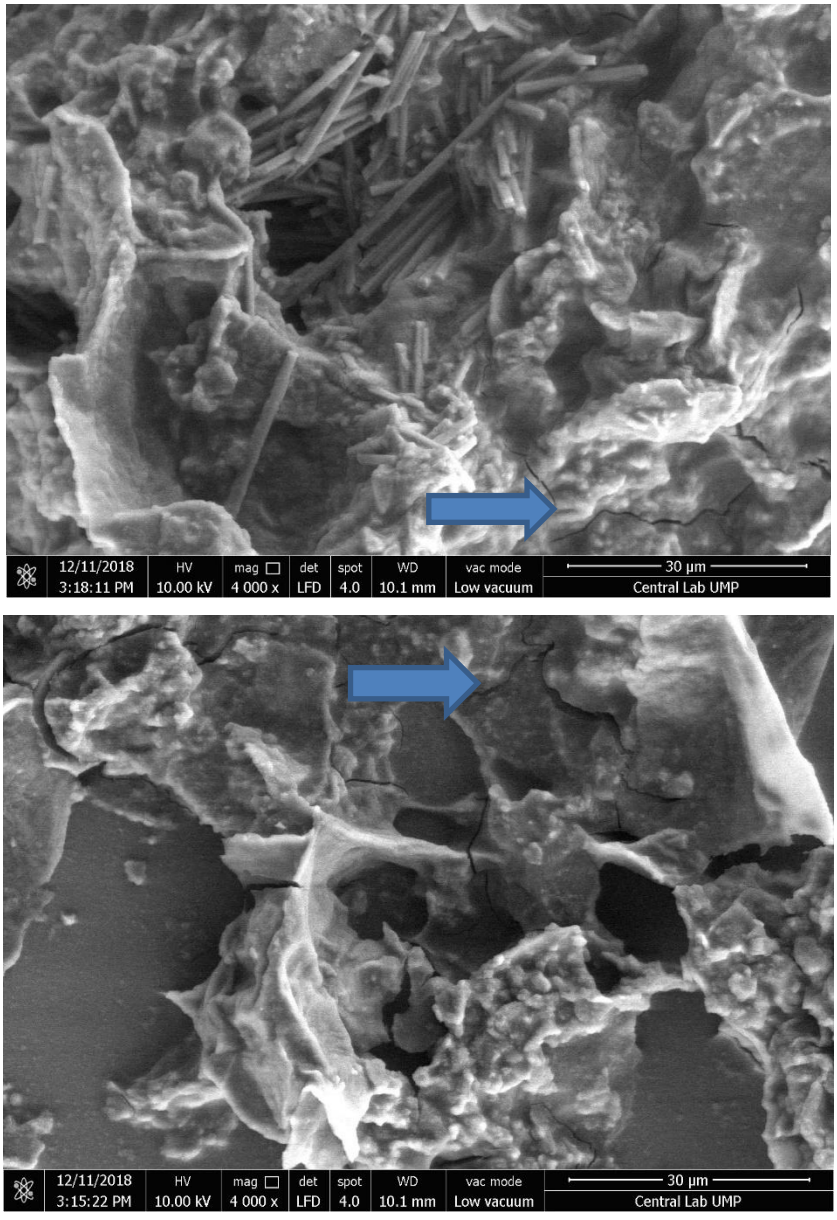


Figure 4.8 Cell structures after 20 minutes of EP treatment

4.5 DISCUSSION

In this project, several options for the period time, T (time per cycle) which from 0.2 seconds to 1.2 seconds was created. The period time are control by giving number's instruction using android Bluetooth software application. Number's instruction was made in the Arduino coding, when the Arduino receive the instruction from the phone then it starts to operate. The idea to create a wireless control circuit and to prioritise the safety of bring this project to Bluetooth based circuit controlling. It is about half way to IOT (Internet of Things) base to keep up with technology nowadays. The Bluetooth module HC-05 was chosen as the component to communicate mobile phone and Arduino board. The indicator light on HC-05 will blink faster when on connection and blink slower to show as connected with mobile phone.

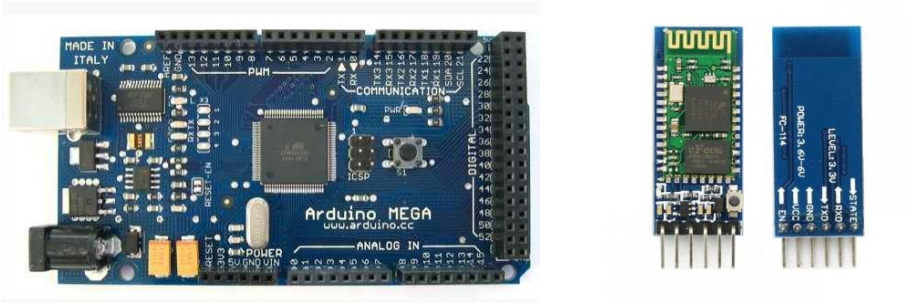


Figure 4.9 Arduino MEGA and Bluetooth module HC05

Bluetooth module HC05 has 2 pin which name TX and RX, it is using to connect with Arduino board to make a communication. The communication makes Arduino doing the instruction by wireless control. The Bluetooth application software give numerical command instruction to operate the Arduino. The Arduino output follow the instruction condition which had programmed in Arduino Software (IDE) and uploaded to Arduino board. Figure 4.10 show the coding made in Arduino Software. In this project, LCD is use as the display to inform user the output in this project. First of all, LCD library must include to activate the LCD for reading coding to display the output. Detail coding and comment can be check at chapter 3.5 for better understanding on each coding.

```
// 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);      // lcd(rs, en, d4, d5, d6, d7)
```

Figure 4.10 Arduino coding include library

The name of each pin that use as output must be declare before using the name in the coding. At void setup part as show in figure 4.11, each pin must be set for its own purpose for example set pin LED1 (pin 31) as output so this pin will give output when HIGH apply to it.

```
// declare the following name and pin number
int PWM = 9;            // PWM output pin at digital pin9
int PWM_out_level;
char data;
int LED1 = 31;
int LED2 = 33;
int LED3 = 35;
int LED4 = 37;
int LED5 = 39;
int LED6 = 41;
int LED7 = 43;          // LEDS at digital pin31,33,35,37,39,41 and 43
int buzzer = 53;        // buzzer to arduino at digital pin 53
int T;

// Set up for the purpose
void setup() {
  Serial.begin(9600);         // set up to start serial display monitor in arduino
  lcd.begin(20, 4);           // set up the LCD's number of columns and rows
  pinMode(PWM, OUTPUT);       // set up PWM which at pin 9 as output
  pinMode(LED1,OUTPUT);       // set up the LEDS at the pin as output
  pinMode(LED2,OUTPUT);
  pinMode(LED3,OUTPUT);
  pinMode(LED4,OUTPUT);
  pinMode(LED5,OUTPUT);
  pinMode(LED6,OUTPUT);
  pinMode(LED7,OUTPUT);
  pinMode(buzzer, OUTPUT);    // set up the buzzer at pin 53 as output
  PWM_out_level = 255;        // set PWM at full speed which at 255
  }
```

Figure 4.11 Declare and setup for each pin

Void loop is where all the instruction take part. In this section, the instruction is keep looping until user stop it. The Bluetooth instruction in this section so when data is received at serial available, the instruction for each data will be keep looping and repeating.

```
// Loop all instruction in this section
void loop() {

// Bluetooth
  if(Serial.available() > 0)      // Send data only when you receive data
  { data = Serial.read();         // Read the incoming data & store into data
```

Figure 4.12 Void loop section

If-Else statement is use to differentiate the instruction output doing in Arduino. Arduino will give output according to the instruction given below the if-else statement when respective data is received. For example, when “data == 1” is received, the LED2 will be switch on since there is a HIGH for LED2 at this section. Output will be given at pulse width modulation (PWM) of full speed at 255 to the transistor and operate the relay in the circuit. The “delay(100)” is the timer delay in Arduino to control the frequency. The value of the timer is in milli second which mean 100ms. The frequency can be calculated

by the formula f = 1 / T, where f is frequency and T is time. When time is 100ms, so the frequency is 10Hz. The “lcd.setCursor and “lcd.print” is to set the LCD to display the following text regarding to the row and column that set at cursor.

```
if(data == '0')
 {
digitalWrite( LED1,HIGH);
digitalWrite( LED2,LOW);
digitalWrite( LED3,LOW);
digitalWrite( LED4,LOW);
digitalWrite( LED5,LOW);
digitalWrite( LED6,LOW);
digitalWrite( LED7,LOW);
digitalWrite(PWM,LOW );
delay(500);

lcd.setCursor (0,2);
lcd.print("time = ");
lcd.print(T=0);
lcd.print("ms     ");
lcd.setCursor (0,3);
lcd.print ("THERE IS NO OUTPUT  ");
  }
```

```
else if(data == '1')
  {
 digitalWrite( LED1,LOW);
 digitalWrite( LED2,HIGH);
 digitalWrite( LED3,LOW);
 digitalWrite( LED4,LOW);
 digitalWrite( LED5,LOW);
 digitalWrite( LED6,LOW);
 digitalWrite( LED7,LOW);
 digitalWrite( PWM, HIGH);
 delay(100);
 digitalWrite(PWM,LOW );
 delay(100);

 lcd.setCursor (0,2);
 lcd.print("time = ");
 lcd.print(T=100);
 lcd.print("ms ");
 lcd.setCursor (0,3);
 lcd.print("BEWARE!HIGH OUTPUT! ");
 }
```

Figure 4.13 If-Else statement

After the coding is done and functioning, circuit was constructed according to figure 4.14 and being tested. Arduino board pin 9 is the PWM output that connected to transistor to act as a switch. When HIGH is given from Arduino, the transistor completes the circuit voltage flow, and vice versa. The relay use to operate the pulse generator.

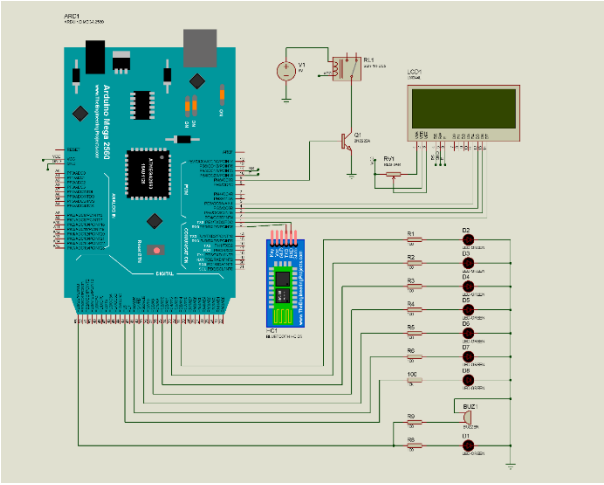


Figure 4.14 Electrical circuit schematic diagram

Circuit was operated by either direct power source from plug or by battery. It is optional at different condition. Battery as source make the product portable and

convenient but only can use for shorter time electroporation treatment, while direct source from plug can use for longer time. If user is doing EP on large amount of sample for a longer time, direct power source from plug is more encouraged to make sure the circuit is always in the same efficiency. Battery source has a shorter life span and only can use for few hours. The current store in the battery also dropped vary with time, it can lower the efficiency of the pulse generator during the electroporation.

User's safety is really prioritised. Few features were added to warn and make user convenient. Bluetooth controlling make sure user stand further from the circuit or contact directly with the circuit during operation. Buzzer and LED indicator light give signal to warn the user the operation is ongoing. Discharging step by touching both terminal of the electrodes must be done after every operation to make sure there is no more voltage in the pulse generator. The remaining voltage will not hurt user, but the spark could cause a little bit pain.

The distance between the electrodes are quite small due to the output probe distance range of the pulse generator. The range is around 0.5cm to 2.0cm and the output will discharge when exceed the range. The output voltage of the pulse generator control by the output probe distance. Distance between the electrodes was fix at 1.0cm during the treatment. At this distance, the cell structure is effectively broke. The transparency of acrylic for the container let user to check and observe the physical changes during treatment. The top cover of the container which can open and close is user friendly. It easier user to wash and clean the container. At the same time, user can place and collect the sample within electrodes easily.

During electroporation treatment, sample was placed in between electrodes to make sure all the sample get treated. Electroporation only occurred between electrodes, the samples which out from the area between electrodes are unusable because there is no electromagnetic field to disrupt the cell structures. Only the particles which took part within electrodes are collected as the samples to observe after electroporation treatment. After 20 minutes of electroporation treatment, the cell wall of the *Pistia* was broke. The cell structures were observed under SEM zooming skill. The broken cell wall prove that electroporation by high voltage square wave pulse is effectively to lignocellulosic biomass.

4.6 COST ANALYSIS

ITEM	COST/ UNIT	QUANTITY	COST (RM)
Arduino MEGA board	RM 41.70/unit	1 unit	RM 41.70
HC-05 Bluetooth Module	RM 25.80/unit	1 unit	RM 25.80
20x4 Liquid-Crystal Display (LCD)	RM 29.50/unit	1 unit	RM 29.50
DC Pulse Generator 6V	RM 14.90/unit	1 unit	RM 14.90
SPDT Relay 6V	RM 5.20/unit	1 unit	RM 5.20
LED light	RM 0.20/unit	10 unit	RM 2.00
Small active buzzer	RM 2.00/unit	1 unit	RM 2.00
Resistor 220Ω	RM 0.20/unit	5 unit	RM 1.00
Transistor 2N2222	RM 7.30/ unit	2 unit	RM 36.00
Battery 6V	RM 15.00	3 unit	RM 45.00
Male to Male Jumper	RM8.80	1 unit	RM8.80
		TOTAL	**RM 211.90**

4.7 GANTT CHART

Research \ Month	Feb'18	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan'19
Title selection and review												
Research on Design												
Finalize Design												
Selection of suitable materials												
Proposal writing												
Proposal presentation												
Submission of final proposal												
Materials Purchasing												
First Prototype - Bluetooth												
Review Prototype - Bluetooth, Relay & Transistor Circuit, Optimizing All circuits												
Finalize Prototype - Soldering, wiring & Testing												
Report preparation												
Submission of report												
Poster preparation & presentation												
Finalized Report												

Sem break

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In this chapter, the conclusion and limitations throughout this project will be discussed. Recommendation is stated to improve this project in the future work.

5.1 CONCLUSION

This project is successfully done under the guidance of supervisor, and supervision from lecturers as well as technical staff together with the help from friends. The design and the fabrication of circuit and reactor that control by Bluetooth is completed and proven that the device can conduct electroporation to treat the lignocellulosic biomass. High voltage square wave pulse which use to conduct electroporation treatment was developed by using Arduino pulse width modulation (PWM) and step up voltage pulse generator. The high voltage output pulse is working according to the PWM from Arduino. PWM is control by the timer delay instruction in the coding. At the same time, frequency of the square wave can be calculated from the timer delay. The relation between time and frequency is inversely proportional which timer increase, frequency decrease and vice versa. As mention before, when HIGH (5V) is given from the Arduino PWM, the pulse generator steps up the voltage and convert it to 30kV high voltage together with the HIGH/LOW PWM square pulse to treat the *Pistia Stratiotes.*

The fabricated reactor includes of the aluminium electrodes and acrylic container. Aluminium sheet was cut into smaller size to use as electrodes because of its higher conductivity that can faster the reaction. The metal use as the electrodes do affect the performance of electroporation. Acrylic was use as the material to make the container

because user can observe the physical changes during the electroporation. For example, during circuit testing, user can see the bubbles occurred on the electrodes which mention at result figure.

The circuit was validated and proved that the *Pistia's* cell structure was disrupted. The dry *Pistia* sample was mashed into small particles and placed within the electrodes and leave for 20 minutes electroporation treatment. The distance between the electrodes is 1cm. The cell structure was disrupted efficiently at this distance due to the strong intensity of the applied high pulse voltage to the treatment medium. After 20 minutes of the electroporation treatment, the broken cell wall was clearly observed compare to the cell structure that did not undergo the treatment. More obvious and effective cell disruption should get for longer treatment time due to the square wave pulse produced varied with time. It was clearly proved from the SEM analysis result. At 0 minute which the sample before treatment, the cell structure look intact. After 10 minutes of electroporation treatment, crack can be seen on the cell structure and much larger and longer crack can observe at 20 minutes treatment time.

In a nutshell, electroporation of lignocellulosic biomass (*Pistia Stratiotes*) is successfully done in this project. As a cost-effective, easy handing and efficient treatment technique, electroporation must be promoted to worldwide especially those agriculture and forestry factory for biomass treatment so its function will not overwhelmed.

5.2 LIMITATIONS OF THE PROJECT

In this project, some challenges and limitations were faced. The device is unable to operate for very long time due to the components overheated. The components could burn down if operate for a longer time. Overheated can reduce the lifespan of components and cause the component to not function well and then affect the performance of the electroporation. Besides that, the distance between the electrodes is limited where ranged at 0.5cm to 2.0cm. When exceed this distance, it will automatically discharge. The distance of the electrodes cause smaller area and less sample can be treat in one time. Other than that, lower frequency that make the treatment to be slower due to the component cannot support and function under higher frequency. When higher frequency is applied means the pulse at every timer will be shorter until the relay cannot support such faster operation. Additionally, Bluetooth as the module to control this device also limited by the connection distance range. User have to leave their mobile phone together with the device during conducting the electroporation treatment. These limitations can be overcome and improve in the future.

5.3 RECOMMENDATION

Few of the recommendations for future work that can be applied to improve this project. After operated for a long time, the device must be switched off to cold down for few minutes before continuing the operation. This can reduce the chance for components to burn and need to change a new one. Small scale of step up DC to DC converter module can be used to replace the pulse generator to create a better high output voltage for electroporation. Larger distance could be done by this module. By increasing the distance, the amount of sample to treat in once also increased. Motor driver module also can be used to replace the relay so that higher frequency can be applied to produce more square pulse in order to faster the electroporation treatment. Extra relay or motor driver module can be added to conduct electroporation treatment on different sample at the same time. Moreover, IOT (Internet of Things) based which can control device at a further distance is applicable to replace the Bluetooth module so user can bring their mobile phone when conducting the electroporation treatment without effect the device. Better outcome can be obtained by applying these recommendation in the future work.

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APPENDIX A

Arduino MEGA 2560 Pin out diagram and specification.

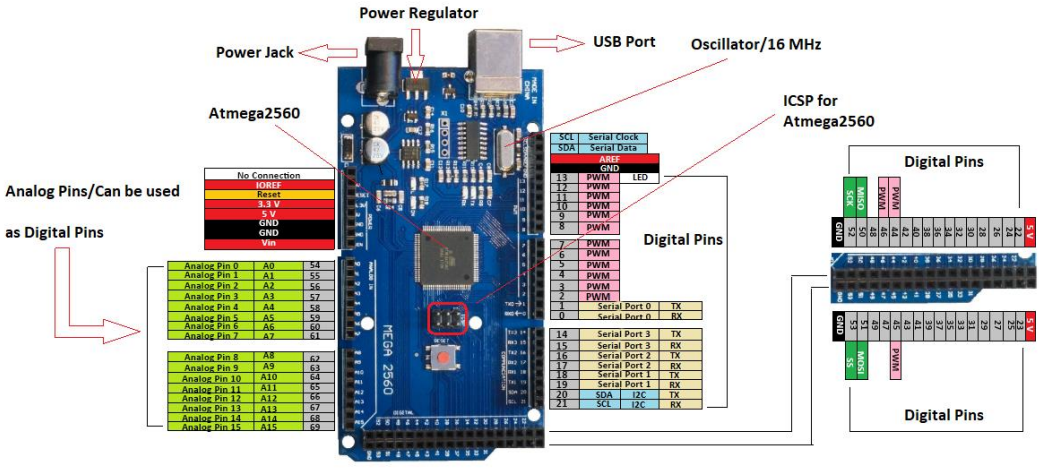


Figure 6.1 Arduino MEGA 2560 Pin out diagram

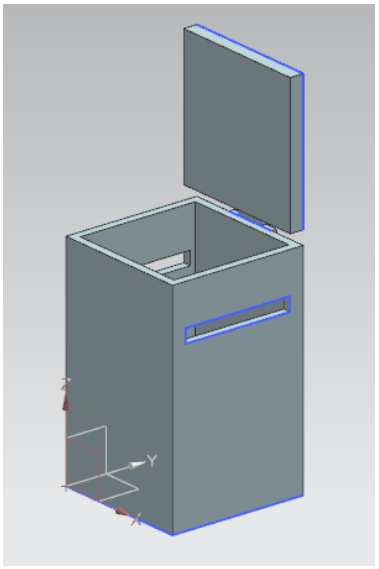
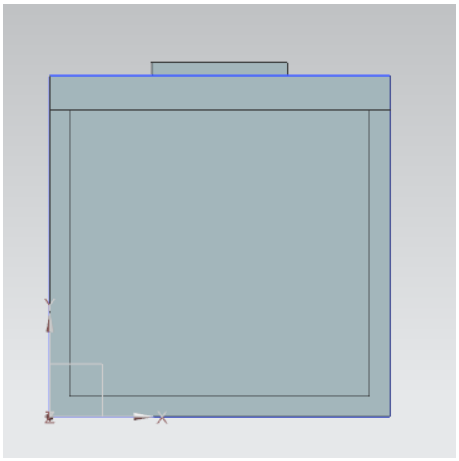
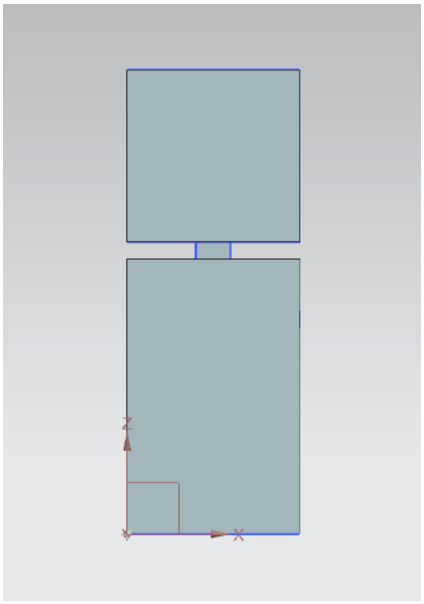
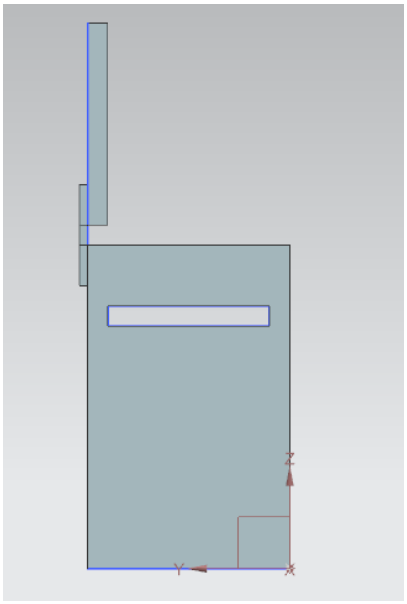
Table 6.1 Specification of Arduino MEGA

Microcontroller	Atmega2560
Operating Voltage	5V
Input Voltage	7V – 12V
USB Port	Yes
DC Power Jack	Yes
Current Rating Per I/O Pin	20mA
Current Drawn from Chip	50mA
Digital I/O Pins	54
PWM	15
Analog Pins (Can be used as Digital Pins)	16 (Out of Digital I/O Pins)
Flash Memory	256KB
SRAM	8KB
EEPROM	4KB
Crystal Oscillator	16 MHz
LED	Yes/Attached with Digital Pin 13
Wi-Fi	No
Shield Compatibility	Yes

APPENDIX B

Electroporation reactor

Table 6.2 Acrylic container mechanical drawing

 Isomeric View	 Top View
 Front View	 Side View

APPENDIX C

Process of project

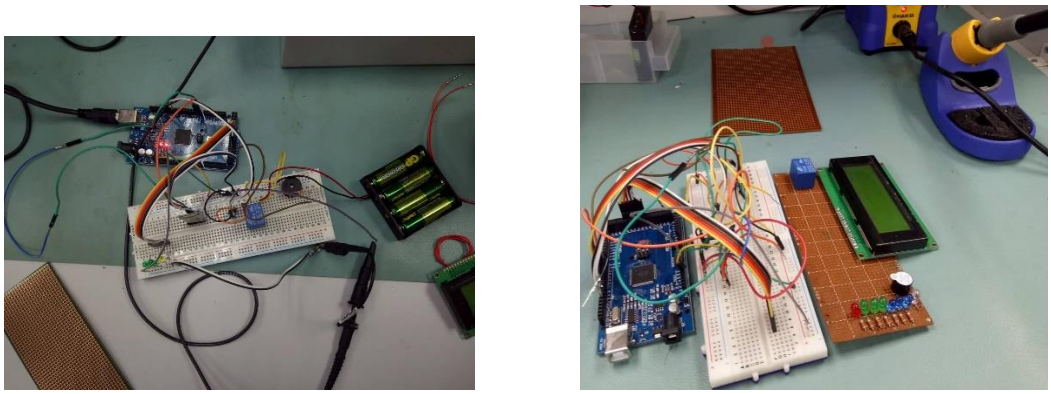


Figure 6.2 Wiring and circuit connection

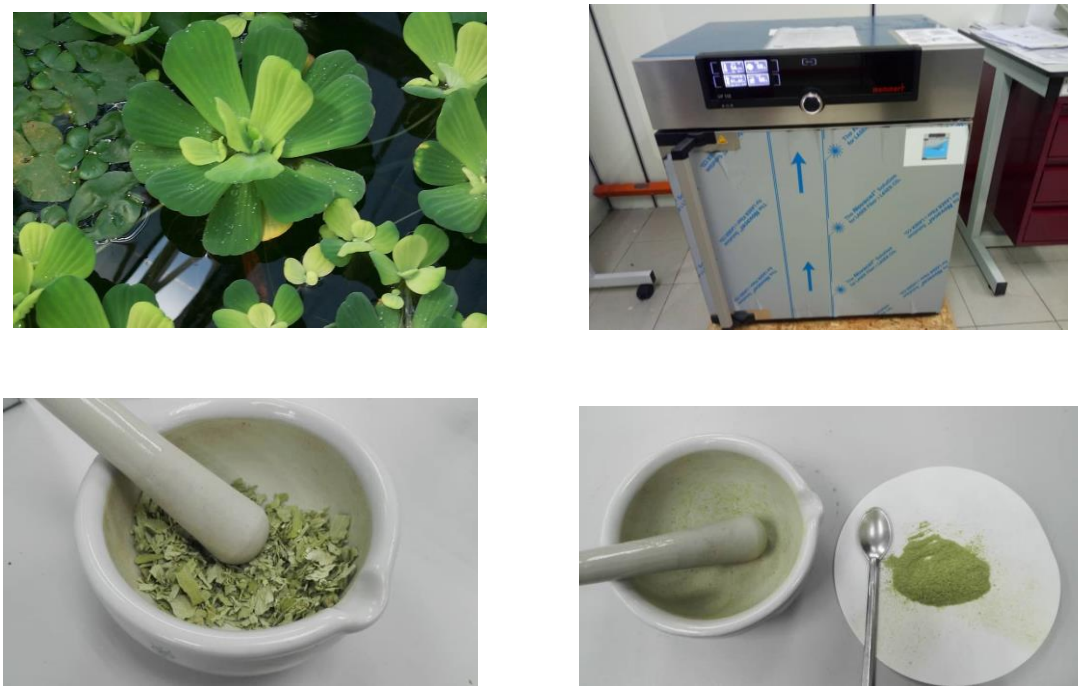


Figure 6.3 Drying of *Pistia* sample

APPENDIX D

Electroporation on Water Lettuce (*Pistia Stratiotes*)

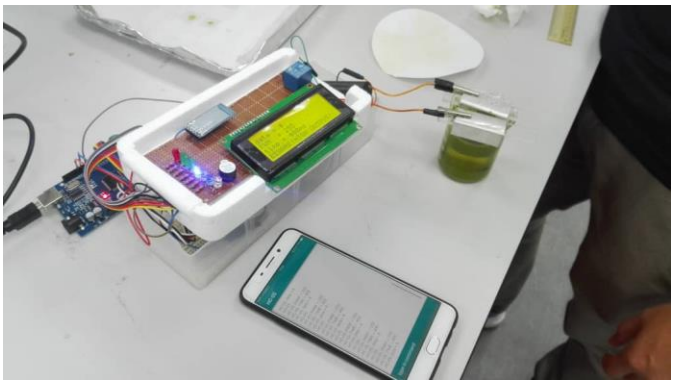


Figure 6.4 Electroporation experiment

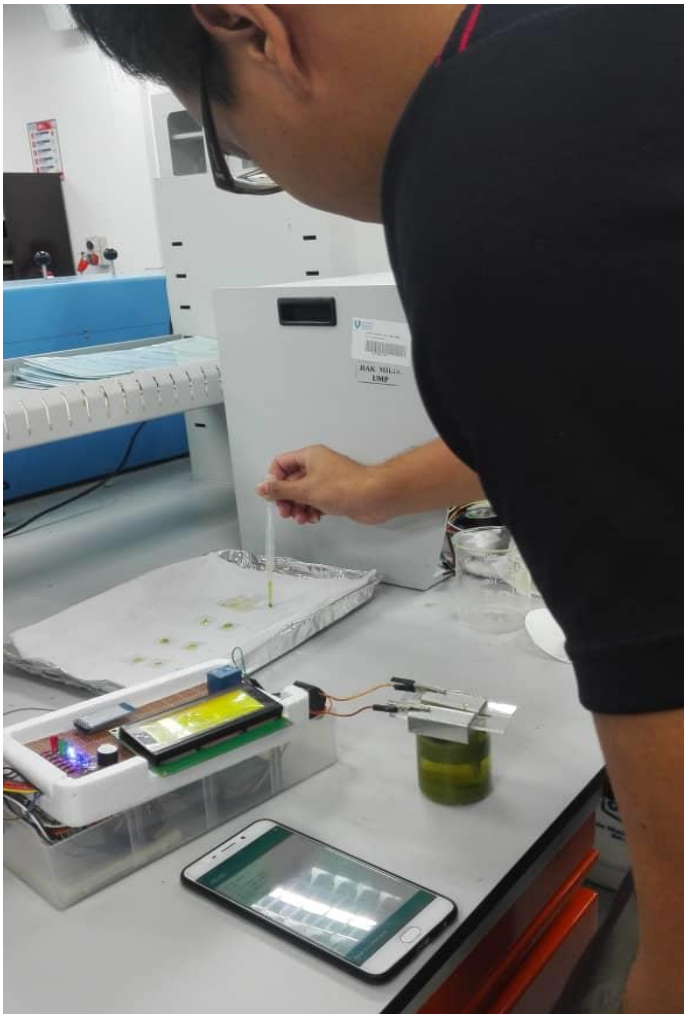


Figure 6.5 Collect sample from mixture

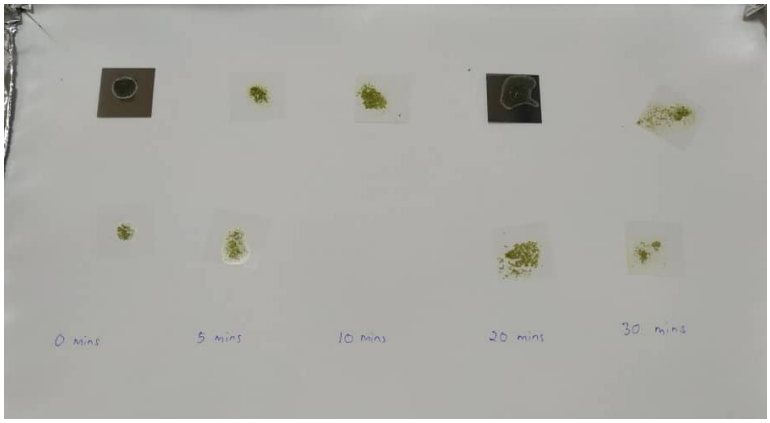


Figure 6.6 Sample collected

Figure 6.7 SEM analysis