ELECTROPORATION OF LIGNOCELLULOSIC BIOMASS

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Thesis submitted in fulfilment of the requirements
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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of degree of Bachelor of Engineering Technology (Manufacturing) with Hons.

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries in which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ABSTRACT

Nowadays, fossil fuels are still the primary energy sources but it cannot hold for a lifetime. Therefore, to overcome this limitation, biofuel is considered as a sustainable solution.A number of methods have been developed for producing biodiesel but with traditional method its take more time and associated with usage of huge amount of toxic solvents. Therefore, our research aimed how to reduce time of lignocellulosic biomass cells breakdown, with less energy input and eco-friendly.This study discussed how to break the bonding of lignocellulosic biomass that make biofuel in a short time using high voltage.As the lignocellulosic biomass are abundant and sustainable feedstock for biofuel production, Azolla was used as source of biomass as it shows greater potential of growth rate. To do this, we used electroporation process. This study produced an electroporation device to deform the cellulosic structure of lignocellulosic biomass to release sugar. Electroporation circuit isfabricated to employed a high voltage for shorter time on lignocellulosicbiomass to disrupt bonding between cellulose, hemicellulose and lignin.A cylinder shape stainless steel with height of 5cm and arc length of 5.3cm is used as the electroporation plate. A voltage within range 1 kV to 4.9 kVhas been used throughout this process. Finally, 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.

ABSTRAK

Kini, bahan api fosil masih menjadi sumber tenaga utama tetapi ia tidak boleh bertahan lama. Oleh itu, untuk mengatasi masalah ini, bahan api bio dianggap sebagai penyelesaian yang terbaik. Beberapa kaedah telah dibangunkan untuk menghasilkan biodiesel, tetapi dengan kaedah tradisional, ia mengambil banyak masa dan menggunakan pelarut toksik yang banyak. Oleh itu, penyelidikan kami bertujuan untuk mengurangkan masa untuk memecahkan sel membran biomass lignoselulosa, dengan input tenaga yang kurang dan mesra alam. Kajian ini membincangkan cara memecahkan ikatan biomas lignoselulosa yang menjadikan biofuel dalam masa yang singkat dengan menggunakan voltan tinggi. Oleh kerana biojisim lignoselulosik adalah bahan makanan yang banyak dan mampan untuk pengeluaran biofuel, Azolla digunakan sebagai sumber biomas kerana ia menunjukkan potensi kadar pertumbuhan yang lebih besar. Untuk melakukan ini, kami menggunakan proses elektroporasi. Kajian ini menghasilkan peranti elektroporasi untuk mengubah struktur selulosik biomas lignoselulosa untuk melepaskan gula. Litar elektroforesis dibuat untuk menggunakan voltan yang tinggi untuk masa yang lebih singkat pada biomassa lignoselulosa untuk mengganggu ikatan antara selulosa, hemiselulosa dan lignin. Keluli tahan karat bentuk silinder dengan ketinggian 5cm dan panjang arka 5.3cm digunakan sebagai plat electroporation. Voltan dalam jarak 1 kV hingga 4.9 kV telah digunakan sepanjang proses ini. Akhir sekali, potensi bahan api bio sebagai salah satu sumber tenaga yang tidak boleh diabaikan kerana ia boleh memberikan sumbangan yang besar untuk membekalkan tenaga pada masa depan.

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LIST OF SYMBOLS

cm	Centimeter
°C	Degree Celsius
g	Gram
kV	Kilovolt
m	Meter
mg	Milligrams
ml	Milliliter
min	Minute

LIST OF ABBREVIATIONS

AC	Alternating Current
DC	Direct Current
EP	Electroporation
PEF	Pulse Electric Field
SEM	Scanning Electron Machine

CHAPTER 1

INTRODUCTION

1.1 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.

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.

In this project, we are using Pistia stratiotes. Pistia is a genus of aquatic plant in the arum family, Araceae. The single species it comprises, Pistia stratiotes, is often called water cabbage, water lettuce, Nile cabbage, or shellflower. Its native distribution is uncertain, but probably pantropical. It was first discovered from the Nile near Lake Victoria in Africa. It is now present, either naturally or through human introduction, in nearly all tropical and subtropical fresh waterways. The genus name is derived from the Greek word πιστός (pistos), meaning "water," and refers to the aquatic nature of the plants.

It is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water, its roots hanging submersed beneath floating leaves. The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures which trap air bubbles, increasing the plant's buoyancy. The flowers are dioecious, and are hidden in the middle of the plant amongst the leaves. Small green berries form after successful fertilization. The plant can also undergo asexual reproduction. Mother and daughter plants are connected by a short stolon, forming dense mats.

1.2 PROBLEM STATEMENT

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.

1.3 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 lignocellulosic biomass (Azolla) by electroporation process.

CHAPTER 2

LITERATURE REVIEW

2.1 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 it controls system is simple, the currents produce is high, have the symmetrical shape associated with a perfect square wave and the time parameter is flexible.

Some researcher was conducting a possibility study about electroporation as a pretreatment method that use algal biomass as feedstock for anaerobic digestion to enhance the production of biogas [10]. Unfortunately, only certain type of cell can withstand the PEF strength. So, a suitable biomass must be choosing thoroughly for the experiments. A cell with a hard cell wall are not effective if using the PEF strength as it will results in the biological membrane disruption which causes the release of intracellular compounds [10]. The membrane became unstable and breaks because of the formation of the pores due the electrical. So, potential and suitable materials of the 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.

2.2 THEORY 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. Application of strong electric field pulses to cells and tissue is known to cause some type of structural rearrangement of the cell membrane [11].. 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.Application of strong electric field pulses to cells and tissue is known to cause some type of structural rearrangement of the cell membrane [12].The transmembrane potential induced in a cell by an external field is generally described by the equation:

$$\Delta V_m = f\, E_{ext}\; r \cos \Phi$$

where Vm is the transmembrane potential, f a form factor describing the impact of the cell on the extracellular field distribution, E_{ext} the applied electric field, r the cell radius and Φ the polar angle with respect to the external field [13].

Some of the studies proposed mathematical models to describe an electroporation process (Joshi and Schoenbach 2000; Krassowska and Filev 2007), and also proposed electrical circuit model to optimize the process. Here, symbols are represented as Vo → the external voltage, Rs → the resistance of the experimental setup, C → the capacitor (the cell’s membrane), R → the resistance of the membrane, Ip → current through electropores. The transmembrane potential, Vm can be expressed by the following differential equation:

$$\mathrm{CVm} + \left(\frac{1}{R_s} + \frac{1}{R}\right) V_m + I_p = \frac{V_o}{R_s}$$

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 trans membrane potential provides the free energy necessary for structural rearrangements of membrane phospholipids and thus enables hydrophilic pore formation.

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, voltage applied, temperature and electroporation buffer.

2.3 MATERIALS INVOLVE IN THE FABRICATION

2.3.1 Stainless Steel

There are many types of materials used to fabricate the electroporation device. All the materials are choose based on the advantages it gives the most. As the electroporation concept is to allows the current through the plates, therefore, a suitable materials that is electrical conductivity must be choose to fabricate the electroporation device.

The electrodes are commonly fabricated out of aluminum (Al), stainless steel, platinum (Pt) or graphite [15]. For the electroporation plates, a stainless steel is used. This is because, the stainless steel have a unique ability to resist corrosion. In addition, the roughness of the stainless steel increase progressively in proportion with the total amount of the electric charge that had passed through the unit area of the electrode [14].

The stainless steel is used in small quantity which is affordable and reasonable. Commonly, a needle shape or small rectangular shape is used for the electroporation plates. But, in this project, a parallel cylinder shape of stainless steel is used as the electroporation plates. Therefore, the plates are shape in half cylinder each. This is because, the cylinder shape give advantage in covered more surface area of the Azolla plant in the process. Mostly of cubic or cylindrical shape and the filling volume between parallel, rectangular, or disc electrodes have been used (Sale and Hamilton, 1967; Neumann and Rosenheck, 1972; Zimmermann *et al.*, 1974; Kinosita and Tsong, 1977; Berg *et al.*, 1984).

i. Stainless steel properties

Corrosion Resistance

All stainless steels are iron-based alloys that contain a minimum of around 10.5% Chromium. The Chromium in the alloy forms a self-healing protective clear oxide layer. This oxide layer gives stainless steels their corrosion resistance. The self healing nature of the oxide layer means the corrosion resistance remains intact regardless of fabrication methods. Even if the material surface is cut or damaged, it will self heal and corrosion resistance will be maintained.

Conversely, normal carbon steels may be protected from corrosion by painting or other coatings like galvanising. Any modification of the surface exposes the underlying steel and corrosion can occur.

The corrosion of different grades of stainless steel will differ with various environments. Suitable grades will depend upon the service environment. Even trace amounts of some elements can markedly alter the corrosion resistance. Chlorides in particular can have an adverse effect on the corrosion resistance of stainless steel.

Work Hardening

Work hardenable grades of stainless steel have the advantage that significant increases to the strength of the metal can be achieved simply through cold working. A combination of cold working and annealing stages can be employed to give the fabricated component a specific strength.

A typical example of this is the drawing of wire. Wire to be used as springs will be work hardened to a particular tensile strength. If the same wire was to be used as a bendable tie wire, it would be annealed, resulting in a softer material.

Hot Strength

Austenitic grades retain high strength at elevated temperatures. This is particularly so with grades containing high levels of chromium and/or high silicon, nitrogen and rare earth elements (e.g. grade 310 and S30815). High chromium ferritic grades like 446 can also show high hot strength.

The high chromium content of stainless steels also helps to resist scaling at elevated temperatures.

Ductility

Ductility tends to be given by the % elongation during a tensile test. The elongation for austenitic stainless steels is quite high. High ductility and high work hardening rates allows austenitic stainless steels to be formed using severe processes such as deep drawing.

High Strength

When compared with mild steels, stainless steels tend to have higher tensile strength. The duplex stainless steels have higher tensile strengths than austenitic steels.

The highest tensile strengths are seen in the martensitic (431) and precipitation hardening grades (17-4 PH). These grades can have strengths double that of TYPES 304 and 316, the most commonly used stainless steels.

Magnetic Response

Magnetic response is the attraction of steel to a magnet. Austenitic grades are generally not magnetic although a magnetic response can be induced in the low austenitic grades by cold working. High nickel grades like 316 and 310 will remain non-magnetic even with cold working.

ii. Stainless Steel Families

Austenitic Stainless Steels

Austenitic stainless steels contain a minimum of 16% chromium and 6% nickel. They range from basic grades like 304 through to super austenitics such as 904L and 6% Molybdenum grades. By adding elements such as Molybdenum, Titanium or Copper, the properties of the steel can be modified. These modifications can make the steel suited to high temperature applications or increase corrosion resistance. Most steels become brittle at low temperatures but the Nickel in austenitic stainless makes it suited to low temperature or cryogenic applications.

Austenitic stainless steels are generally non-magnetic. They are not able to be hardened by heat treatment. Austenitic stainless steels rapidly work-harden with cold working. Although they work harden, they are the most readily formed of the stainless steels. The principal alloying elements are sometimes reflected in the name of the steel. A common name for 304 stainless steel is 18/8, for 18% chromium and 8% nickel.

Ferritic Stainless Steels

Ferritic stainless steels include grades like 430 and contain only chromium as a major alloying element. The quantity of chromium present ranges from 10.5 to 18%. They are known for their moderate corrosion resistance and poor fabrication properties. Fabrication properties can be improved by alloy modifications and are satisfactory in grades such as 434and 444. Ferritic stainless steels cannot be hardened by heat treatment and are always used in the annealed condition. Ferritic stainless steels are magnetic. They are also not susceptible to stress corrosion cracking. Weldability is acceptable in thin sections but decreases as section thicknesses increase.

Martensitic Stainless Steels

High carbon and lower chromium content are the distinguishing features of martensitic stainless steels when compared with ferritic stainless. Martensitic stainless steels include 410 and 416. Hardened martensitic steels cannot be successfully cold formed. They are magnetic, have moderate corrosion resistance and poor weldability.

Duplex Stainless Steels

Duplex stainless steels have high chromium and low nickel contents. This gives duplex stainless steels microstructures that include both austenitic and ferritic phases. They include alloys like 2304 and 2205. These alloys are so named due to their respective compositions - 23% chromium, 4% nickel and 22% chromium, 5% nickel.

By having both austenite and ferrite in the microstructure, duplex stainless steels feature properties of both classes. Although a compromise between the two ‘pure’ types, duplex grades can offer some unique property solutions. Duplex grades are resistant to stress corrosion cracking, but not to the same level as ferritic grades. The toughness of duplex grades is superior to that of the ferritic grades – but inferior to that of the austenitic grades.

Most importantly, the corrosion resistance of duplex steels is equal, or superior to 304 and 316 stainless steel. This is particularly so for chloride attack.Duplex grades are readily welded. They also have high tensile strengths.

Precipitation Hardening Grades

Precipitation hardening grades contain both Chromium and Nickel. They develop very high tensile strengths with heat treatment. Precipitation hardening grades are usually supplied in a “solution treated” condition that allows the steel to be machined. After machining or forming, the steel can be aged in a low temperature heat treatment process. As the heat treatment is performed at low temperatures, no distortion is induced in the work piece.

2.3.2 Lid

For the lid, a transparent acrylic plate is used. The main function of lid is to hold the electrodes and grantee the flow of current to the parallel cylindrical stainless steel plates. The main advantage of using a transparent acrylic plate is because we can observe the electroporation process from above (ectroporation plate). Acrylic is a plastic manufactured using one or more derivatives of acrylic acid. Polymethyl Methacrylate acrylic, or PMMA, is one of the more widely used forms of acrylic due to its exceptional weatherability, strength, clarity and versatility. Besides, it also easily fabricated and shaped, the weight is also less 50% than glass which making it more easier to handle.

From the view of safety, acrylic plate is electrical insulation. Therefore, during the electroporation process, the lid is free to be touched. Therefore, accidentally touched will not give any harm to the user.

2.3.3 Cylindrical Rod Metal

Cylinderical rod metal is used to hold the electroporation plates which is the parallel cylinder shape of stainless steel and to supply the current to the plates at the other end. The cylindrical rod metal is the common metal used just to connect both the plates and the lid. Therefore, the safety will be more secure while working on the electroporation process.

2.4 MACHINING INVOLVE IN FABRICATION

During the fabrication of the electroporation device, various of machine are used in the process. Firstly, a hand cutting machine is used to cut the cylinder stainless steel.

2.4.1 Hand Cutting Machine

The first metal-cutting machine tools, builts some 450 years ago, were powered by water and employed iron and carbon steel tools (David A. Stephenson *et al.*, 2016). A cutting tool or cutter is any tool that is used to remove material from the work piece by means of shear deformation. As the plates is in the half cylinder shape, the cylinder stainless steel must be cut to half first. In the theory of the electroporation, the plates cannot touch each other, therefore, it must be cut more to turn to the desire shape, size and gap. To avoid any accidental cut of the material, a hand grinding machine is used to smooth the surface and remove the metal in a little amount.

2.4.2 Hand Grinding Machine

A grinding machine, often shortened to grinder, is any various power tools or machine tools used for grinding, which is a type of machining using an abrasive wheel as the cutting tool. Hand grinding machine is used to the surface of the cylinder stainless steel that had been cut earlier. It is beneficial for humans in time wise, doing the jobs that needs to be done in short periods of time. The accuracy in dimensions in grinding is of the order of 0.000025mm, in most applications it tends to be a finishing operation and removes comparatively little metal, about 0.25mm to 0.50mm depth.

2.4.3 Hand Drilling Machine

Hand drilling machine is used on the transparent acrylic plate. Drilling operations are operations in which holes are produced or refined by bringing a rotating cutter with cutting edges at the lower extremity into contact with the workpiece (Hudson A.L *et al.*, 1998). Therefore, to make a connection to the power supply, a holes must be drilled. As the power supply can go through the transparent acrylic plate directly as it is an electrical insulation.

2.4.4 Welding

Welding is a fabrication or sculptural processes that join materials, usually metals or thermoplastics, by causing coalescence. It is also can be defined as a process in which materials of the same fundamental type or class are brought together and cause to join through the formation of primary chemical bonds under the combined action of heat and pressure (Messler, 1993).

The definition found in ISO standard R 857 (1958) state, “ Welding is an operation in which continuity is obtained between parts for assembly, by various means”. The Welding Institute (commonly known TWI) simply states “e duobus unum,” which means “from two they become one.”

Welding is the act of joining two pieces of metal together. It is also a fabrication or sculptural process that joins materials, usually metals or thermoplastics, by causing fusion, which is distinct from lower temperature metal-joining techniques such as brazing and soldering, which do not melt the base metal.

In addition to melting the base metal, a filler materials is often added to the joint to from a pool of molten materials (the weld pool) that cools to form a joint that can be as strong, or even stronger, than the base materials. A variety of types of welding exist for use in different applications and for the range of metals used in manufacturing.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This chapter will cover the details explanation of methodology that is being used to make this project completed. The method is used to achieve the objective of the project that will accomplish. To evaluate this project, the methodology is based on fabricated the electroporation device. This project used four major steps to implement project starting from planning, implementation and analysis. All the methods used for finding and evaluating data regarding the project related.

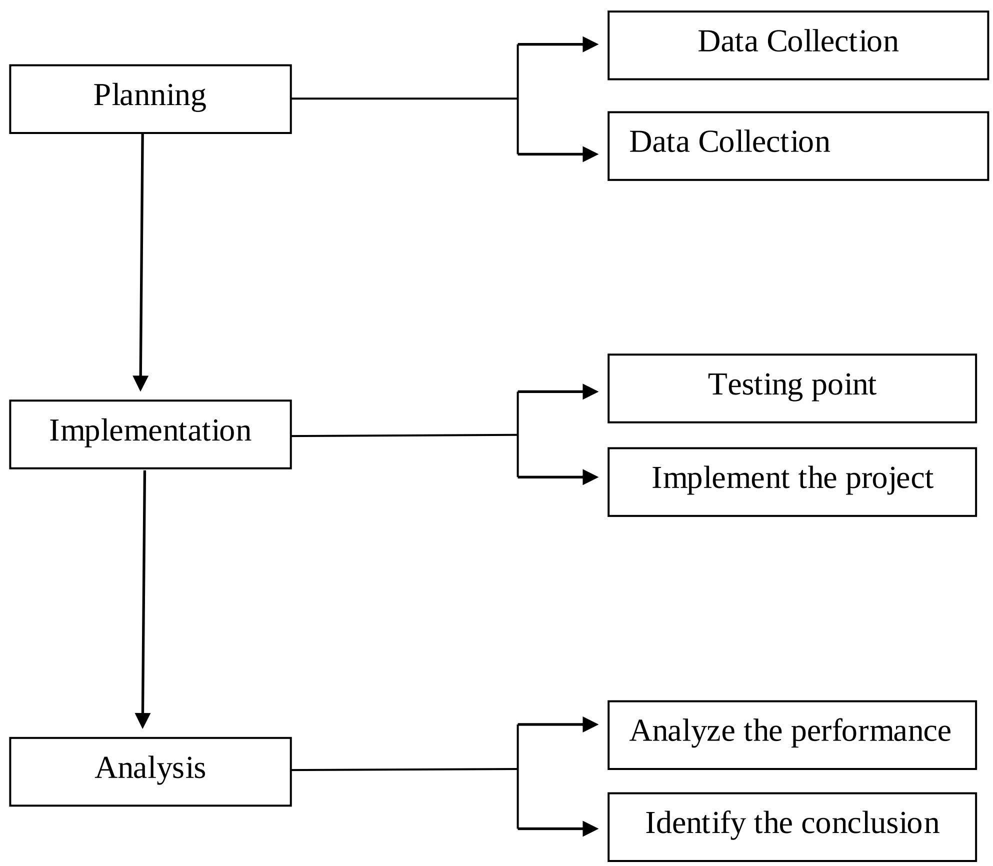

Figure 1: Major steps to implement project

3.2 FLOWCHART OF METHODOLOGY

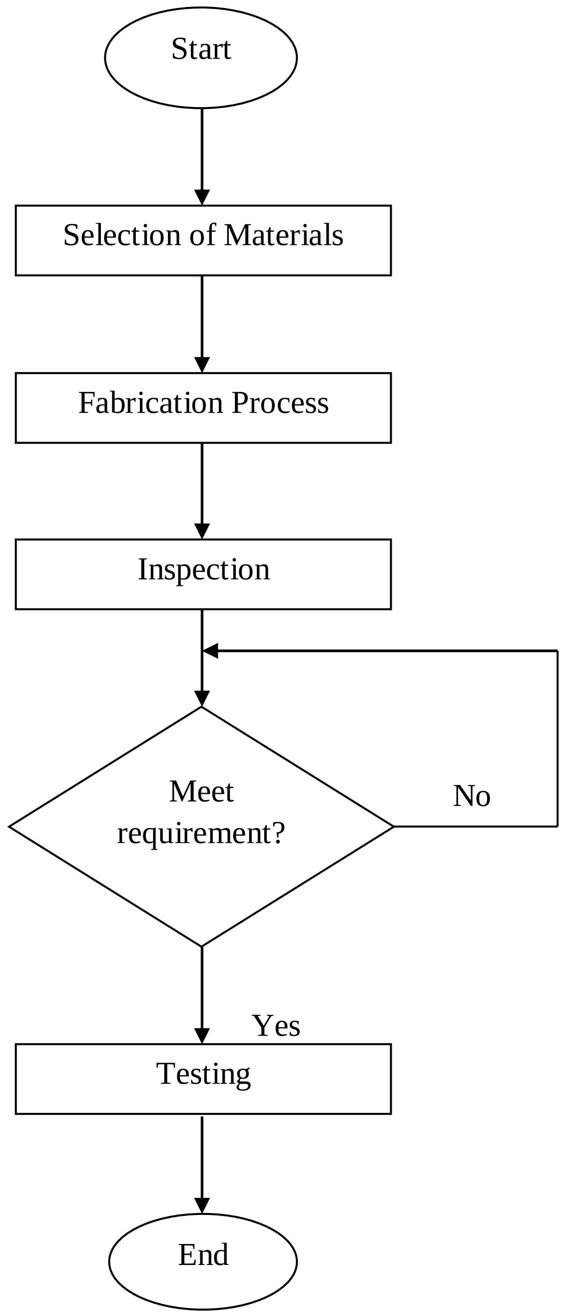

Figure 2: Project Fabrication Process Flowchart

3.3 SELECTION OF MATERIALS

There are several requirement to determine the suitable materials for this electroporation device. By selecting incorrect materials to make the electroporation device, the probability of it not function properly is higher. The primary function of the parts. The requirement choosing materials for the parts includes.

i. The materials for the plate must be electrical conductivity

The electoporation plates must be electrical conductivity in order to supply the current from the power supply to the Azolla plant. Besides, the process took places in water presence and must be test several times to get the actual results. Therefore, a metal that have ability to resist corrosion is a better choice. In this case, a stainless steel is choose for the electroporation plates.

ii. Electrical insulation materials must be choose for the lid

Materials like plastic, for instance, are well known as their ability for electrical insulation. Transparent acrylic plate is chosen to make the lid. Figure below show the example of acrylic plate. Acrylic plate is high performance, a sustainable and eco-efficient material used in a large variety of everyday applications. It has a unique combination of properties, offering clarity, durability, safety and versatility, as well as heat. The table below shows the physical properties of acrylic plate.

Physical Properties	
Density (ρ)	1.20 – 1.22 g/cm^3
Abbe number (V)	34.0
Refractive index (n)	1.584 – 1.586
Flammability	V0 – V2
Limiting oxygen index	25 – 27 %
Water absorption – Equilibrium (ASTM)	0.16 – 0.35%
Water absorption – over 24 hours	0.1%
Radiation resistance	Fair
Ultraviolet (1-380nm) resistance	Fair

Table 1: Physical properties of acrylic plate

Figure 3: Acrylic plastic Sheet (Kapoor, 2015)

3.4 FABRICATION PROCESS OF ELECTROPORATION DEVICE

3.4.1 Drawing of Electroporation Deviced

The design of the electroporation device is done by using the software of NX Nastran 10. It is used, among other tasks usually for 3D design (parametric and direct solid/surface modelling)Nastran is a finite element solver for stress, vibration, buckling, structural failure, heat transfer, acoustics and aeroelasticity analyses.

For the electroporation device, the design was choose based on the advantages it gives for this project which is the electroporation of lignocellulosic biomass. The design consists of 1000 ml beaker with a lid. The main part of the design is actually is the lid. The lid will have holes for the installation of stainless steel electrodes and on top of the lid is the connection to the power supply. Therefore, from the lid that connects to the stainless steel electrodes, an insulated crocodile clip for the continuation to the source of electricity.

Therefore, a software of NX Nastran 10 is used to draw the design in 3D image. So, the image would be easier to analyse while discussing with supervisor to ask for advice for improving the design. Besides, it will be more efficient to ask help with lecturer assistant for the materials used in the project design with a 3D drawing. More ideas and advices regarding the project can be achieved easily.

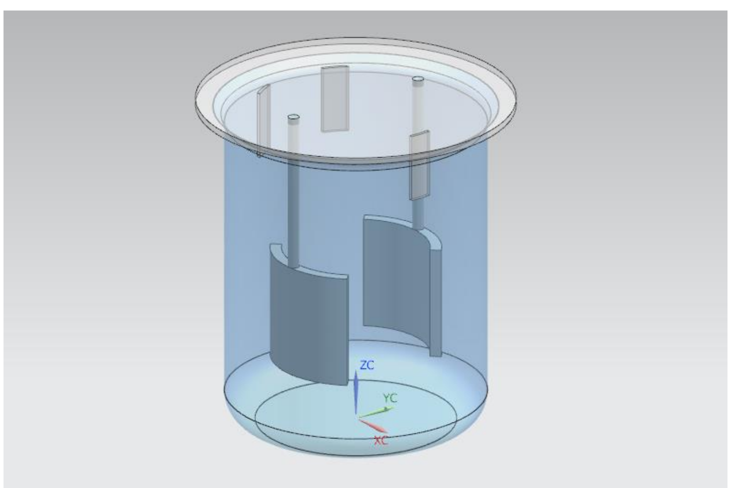

Figure 4: 3D drawing

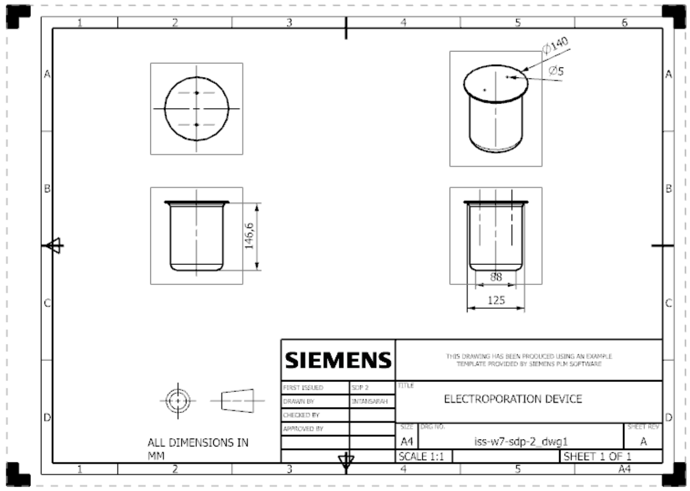

Figure 5: Drawing with measurement

3.4.2 Fabrication Of Electroporation Deviced

For the fabrication of the electroporation device, some machines are used to complete the process to produce the device. There are many types of machines with their own functions to produce the parts that desire. The example is such as the Cutting machine, Hand Cutting machine, Hand Grinding machine, Hand Drilling machine and others.

Firstly, for the stainless steel electrodes, the cylindrical shape metal will be cut first to the desirable size (half cylinder) in two exact pieces. Then, both of the metal will connect to the stainless steel rods. The purpose of using the stainless steel rods is to attach on the lid to get connection to the power supply. Therefore, to make both stainless steel pieces and rods connect to each other, a welding must be done.

Next, to hold the rod to the lid, the end of the rod will connect to a screw shape. Therefore, on the lid, two holes will be done by the Hand Drilling machine. The screw shape will go through the holes and will be secure by the screw nuts. Thus, an insulated crocodile clip will be connected at the screw shape which the power supply can go through it.

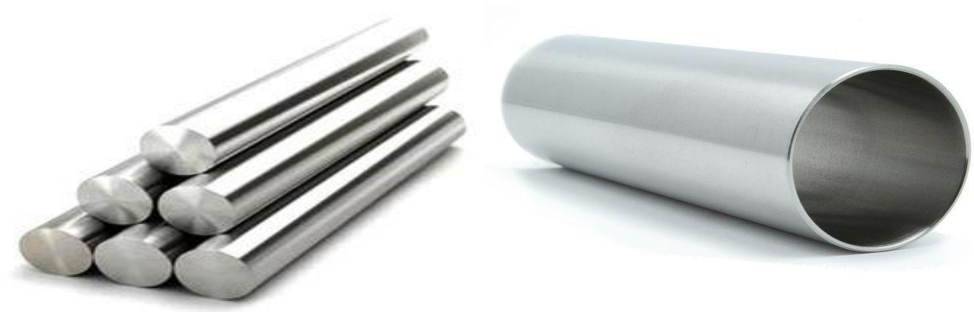

Figure 6: Cylinder rod metal and stainless steel cylinder shape

Figure 7: 1000 ml beaker

Figure 8: Transparent acrylic:

Besides, the machines that involve to fabricate the design is the cutting machine which will use to cut the stainless steel to two pieces. After the cylinder stainless steel metal is cut into two pieces, then, again a hand cutting machine is used to get the desire measurement which is the arc is 30 mm and the height is 50 mm (30 mm x 50 mm).

Figure 9: Cutting machine

Figure 10: Hand grinding machine

Figure 11: Hand cutting machine

Figure 12: Hand drilling machine

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 LIGNOCELLULOSIC BIOMASS COLLECTION

In this project, lignocellulosic biomass that will be use is water lettuce or the scientific names is *pistia stratiotes* in arum family. The sample is collect from a lake in Gambang Resort Water Park located at Gambang, Kuantan. Total amount will be collect are about 500 gram. As preparation for electroporation, the sample will wash the water lettuce and cut into small pieces. Then will put the sample into the reactor.

4.2 ELECTROPORATION TREATMENT

The electroporation process of the water lettuce will be run in three phase within 15 minutes at the range of 5 minute in a 1000 ml beaker. Inside the beaker, will be put about 50 gram of water lettuce that been cut into small pieces. The voltage use is between 4 kV until 10 kV and with 0.0033 Hz for the 5 minutes, 0.0017 Hz for the 10 minutes and 0.0011 Hz for the 15 minutes. After every cycle of electroporation, the sample will be divide into several part for biomass analysis purposes that areField Emission Scanning Electron Microscope (FESEM), hydrolysis, sugar analysis and gas cinematography in order to measure the results.

4.3 BIOMASS ANALYSIS

4.3.1 Scanning Electron Microscope (SEMEDX) TM3030PLUS

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the sample's surface topography and composition. The electron beam is scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image. SEM can achieve resolution better than 1 nanometer. Specimens can be observed in high vacuum in conventional SEM, or in variable pressure or environmental SEM, and at a wide range of cryogenic or elevated temperatures with specialized instrument.

The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons that can be detected depends, among other things, on specimen topography. By scanning the sample and collecting the secondary electrons that are emitted using a special detector, an image displaying the topography of the surface is created.

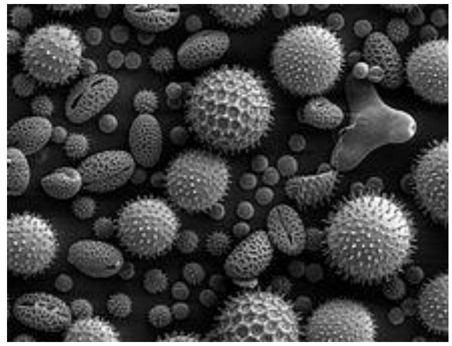

Figure 13: The example of photo of pollen grains taken with SEM

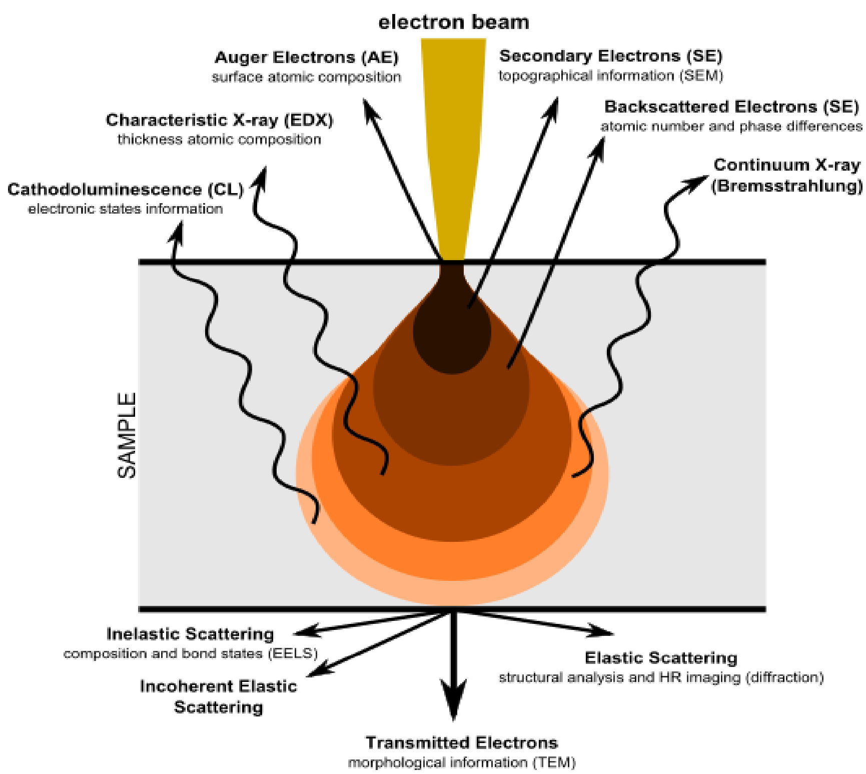

Figure 14: SEM opened sample chamber

4.4 RESULTS

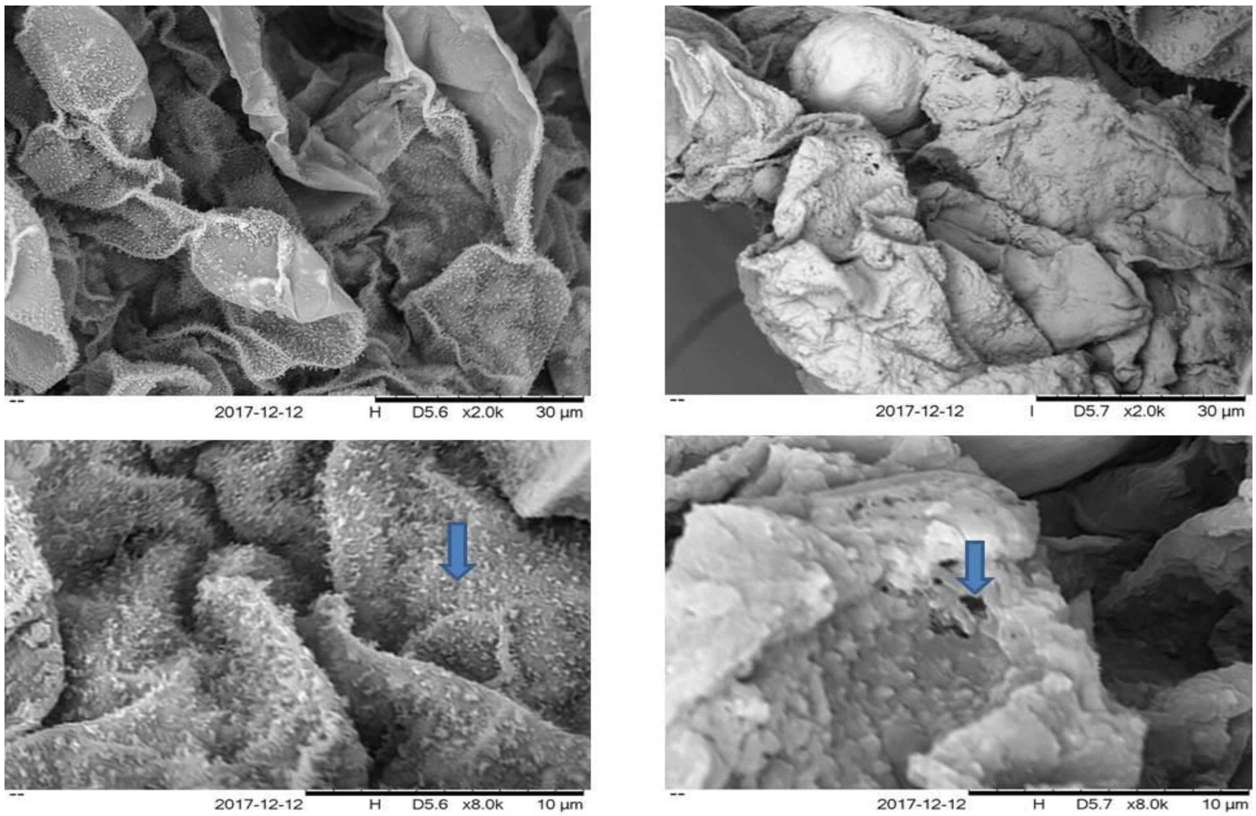

Figure 15: Azolla plant before (above) and after (below) electroporation process

Before and after the electroporation process, the Azolla plant is taken for two samples. Therefore, there are one sample before and one sample after the electroporation proces. This sample is then taken to be scanned using the SEM. From the Figure , the above pictures is the Azolla plant before the electroporation process. The surface of Azolla looks like thorn and not smooth. The Azolla surface still in a good condition where there are no damage occurs to the cell membrane. From the figure, the below pictures the After electroporation process, the surface of Azolla looks smooth and got holes because the cell wall was destroyed.

After electroporation process, we put a small piece of Azolla over glass slide with 2cm x 2cm size and evacuated it for 20 minutes before using the SEM EDX. We used SEM EDX through Tabletop Microscope TM3030Plus. TM3030Plus has a premium SE detector and a BSE detector which have been incorporated in FE-SEM & VP-SEM and well-accepted as a high-sensitivity detector. We used resolution 2000 and 8000 to observe the effect of electroporation process. SEM provides detailed high resolution images of the sample by rastering a focussed electron beam across the surface and detecting secondary or backscattered electron signal.

Figure 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.

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

The reason for the implementation of pretreatment is to open up the biomass structure and break down the lignocellulosic bonding in order to promote enzymatic accessibility to cellulose and hemicellulose for hydrolysis.

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.

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 biogas produced will be more.

5.2 RECOMMENDATION

As the recommendation, the electroporation device can be upgrade in the ways of safety, quality and creativity. Besides, the electroporation process also can be test with different types of electrodes (metal), to compare with the stainless steel to show the best results. Lastly, the lab 1000ml glass beaker can be replace with our own ideas.

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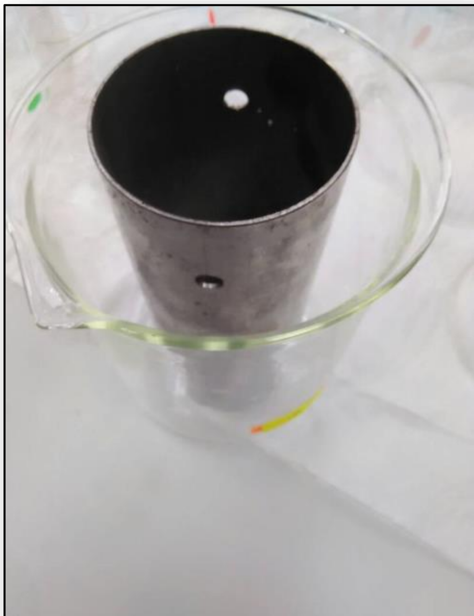
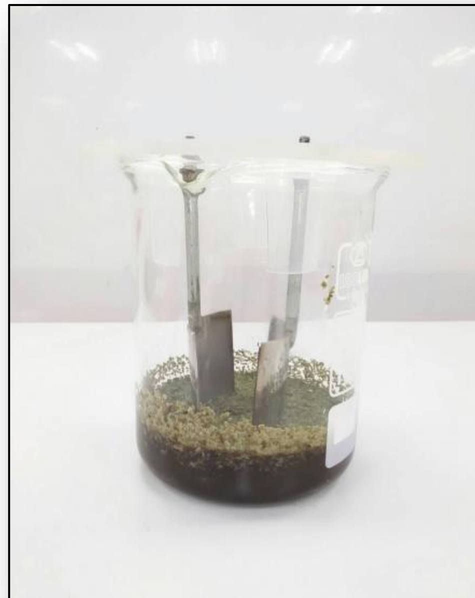
APPENDIX

APPENDIX A

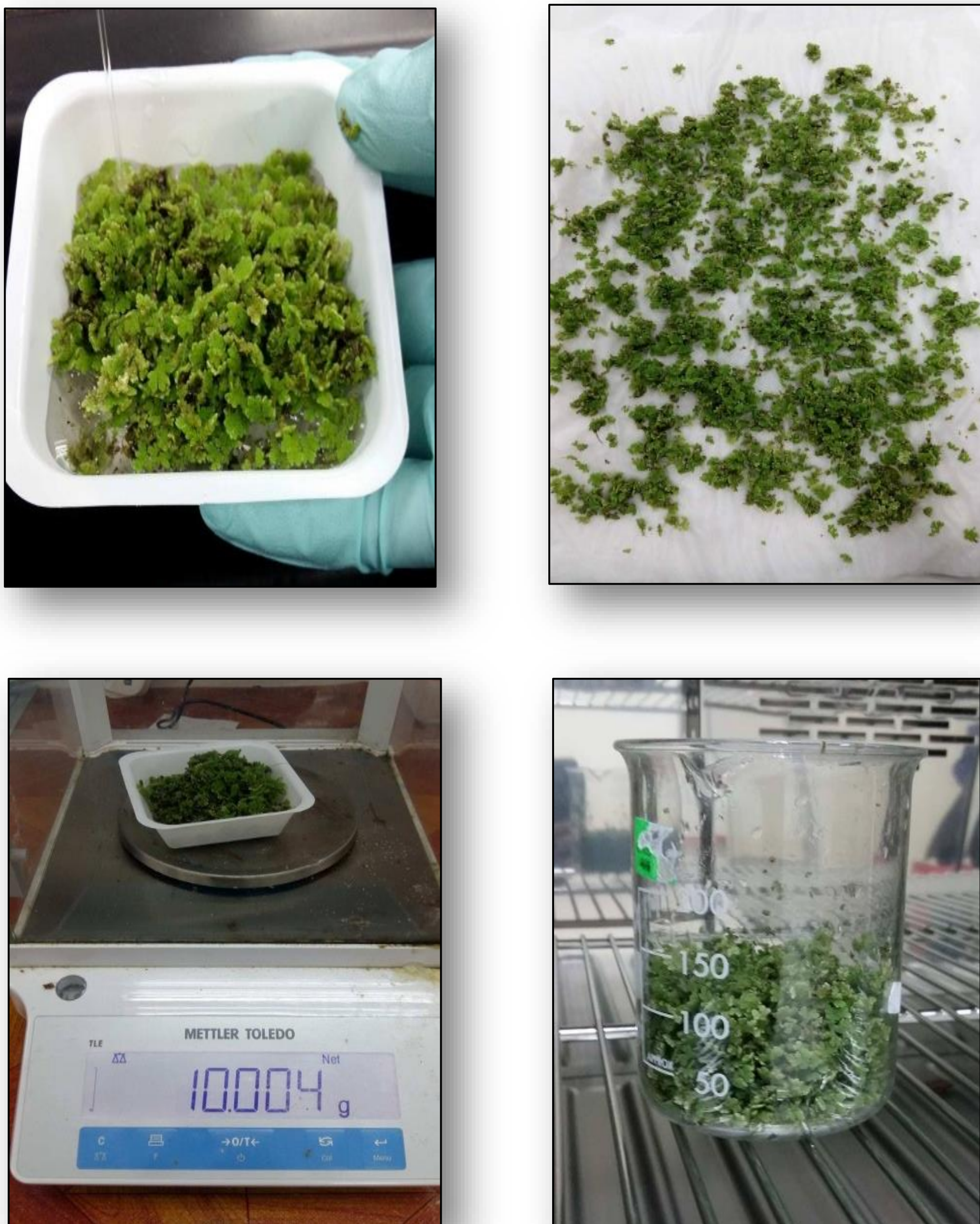
NO.	MATERIALS	COST/UNIT	QUANTITY	COST
1	9V Battery	RM 8.00	4 unit	RM32.00
2	9V DC Connector	RM 3.00	2 unit	RM6.00
3	Voltage regulator	RM7.00	2 unit	RM14.00
4	High Voltage Multiplier 1.0 - Complete Kit Includes PCB Board, Components, and Schematic	RM193.64	1 unit	RM193.64
5	Single wire:- -black (5m) -red (5m) -green (5m)	RM 3/meter	15m	RM 45.00
			Total	RM280.64

Process of the fabrication of the electroporation device





Process of pretreatment



Electroporation of lignocellulosic biomass

APPENDIX B

Research Activities	Month											
	Feb ‘17	Mar ‘17	Apr ‘17	May ‘17	Jun ‘17	Jul ‘17	Aug ‘17	Sep ‘17	Oct ‘17	Nov ‘17	Dec ‘17	Jan ‘18
Title selection and review												
Selection of suitable materials												
Proposal writing												
Proposal revision												
Submission of proposal												
Proposal presentation												
Minor Report writing												
Supplier Discussion												
Revision of minor report												
Submission of minor report												
Thesis preparation												
Thesis revision												
Project Implementation												
Submission of thesis												
Project presentation												

Legend:

- Semester 2 16/17
- Semester Break
- Semester 1 17/18
- Progress

Table 6: Some important dates to highlight:

Date	Highlights
12/5/17	Presentation for Group Report 1.
27/5/17	Submission of Logbook, peer evaluation and Minor Report.
June ‘17 – July ‘17	Purchasing of Materials.
August ‘17– October ‘17	Project Design and Implementation.
November ‘17– December ‘17	Preparation for Thesis and Group Report 2.
15/12/17	Submission of peer evaluation for SDP 2
17/12/17	Extended Report submission
18/12/17	Project Presentation
2/1/17	Submission of Thesis and Logbook for SDP 2