SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING

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CHARACTERIZATION, DELIGNIFICATION AND HYDROLYSIS OF MALAYSIAN ELEPHANT GRASS FOR THE RECOVERY OF SUGAR

By

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DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled "**Characterization, Delignification and Hydrolysis of Malaysian Elephant Grass for the Recovery of Sugar**". I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title for any other examining body or university.

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LIST OF ABBREVIATIONS

SYMBOLS

˚C	**D**egree **C**elcius
%	**P**ercentage
g	**G**ram
ml	**M**illilitre
min	**M**inute
hrs	**H**our
M	**M**olarity
V	**V**olume
W	**W**eight

ABBREVIATIONS

SEM	**S**canning **E**lectron **M**icroscope
RMM	**R**elative **M**olecular **M**ass

CHEMICAL FORMULA

H_2SO_4	Sulphuric Acid
NaOH	Sodium Hydroxide

PENCIRIAN, DELIGNIFIKASI DAN HIDROLISIS RUMPUT GAJAH MALAYSIA BAGI MENDAPATKAN GULA

ABSTRAK

Kenaikan harga minyak mentah dan kejatuhan nilai rizab bahan api fosil di seluruh dunia menjadi salah satu halangan yang paling sukar yang mesti dihadapi pada zaman moden ini ditambah dengan pemanasan global yang tidak dapat dielakkan yang boleh memberi kesan buruk kepada alam sekitar disebabkan oleh peningkatan penggunaan petroleum. Walau bagaimanapun, untuk menjamin pemeliharaan alam sekitar dan pengunaan tenaga bersih, usaha mencari tenaga alternatif adalah sangat penting. Lignoselulosa merupakan salah satu bahan mentah yang menghasilkan gula kerana ia merupakan sumber yang banyak yang boleh diperbaharui dan mempunyai status bukan makanan yang tidak bersaing dengan pengeluaran makanan. Rumput gajah Malaysia (*Pennisetum purpureum*, Schumach.) (USM Herbarium 11741) juga dikenali sebagai rumput Napier adalah salah satu daripada bahan lignoselulosa yang mempunyai potensi yang amat besar untuk pengeluaran polimer karbohidrat di negara-negara tropika seperti Malaysia kerana keupayaan rumput itu menyesuaikan diri dan ketersediaan dalam sesusatu iklim. Dalam kajian ini, ciri-ciri fizikal rumput ditentukan dengan menggunakan SEM dan diperiksa permukaan morfologi sebelum dan selepas proses delignifikasi. Ia menunjukkan bahawa permukaan morfologi mempunyai struktur licin, padat dan seragam. Seterusnya, kecekapan pra-rawatan beralkali dan berasid ditentukan oleh larutan NaOH dibandingkan dan larutan H_2SO_4. 10g sampel dengan saiz 1 cm x 1 cm direndam dengan 1 M larutan dan dipanaskan pada suhu 70˚C dalam masa 2 jam. Ia menunjukkan bahawa larutan NaOH mempunyai kecekapan yang lebih baik berbanding dengan larutan H_2SO_4 dengan penurunan berat sebanyak 60.87%. Keadaan terbaik untuk

delignifikasi rumput turut diuji. Semua faktor (kepekatan, nisbah pepejal kepada cecair dan suhu) sedang diuji untuk menentukan sama ada kesan pemulihan gula. Ia menunjukkan bahawa semua parameter mempengaruhi pemulihan gula.

CHARACTERIZATION, DELIGNIFICATION AND HYDROLYSIS OF MALAYSIAN ELEPHANT GRASS FOR THE RECOVERY OF SUGAR

ABSTRACT

The price rising of crude oil and the depreciation of worldwide fossil fuel reserves become one of the toughest obstacle that must be confronted in this modern era plus with the alarming of unavoidable global warming that gives an awful impact to the environment due to increase in utilization of petroleum. However, to guarantee of environmental protection and energy security, the search of alternative energy is essential. Lignocellulosic are promising raw materials for sugar production since they are abundant renewable resources and its non-food status that not compete with food production. Malaysian elephant grass (*Pennisetum purpureum*, Schumach.) (USM Herbarium 11741) also known as Napier grass is one of a lignocellulosic material that has tremendous potential for carbohydrate polymers production in tropical countries like Malaysia due to their adaptability and availability. In this study, the physical characteristics of the grass were first determined by using SEM and examined the surface morphology before and after the process of delignification. It shows that the surface morphology has smooth, compact and homogeneous structure. Next, the efficiency of alkaline and acidic pretreatments was determined by compared NaOH solution and H_2SO_4 solution. 10g of the sample with size 1 cm x 1 cm is soaked with 1 M of solution and heated at 70˚C in 2 hours. It shows that NaOH solution has better efficiency compare to H_2SO_4 solution with 60.87% weight loss. All parameters (concentration, solid to liquid ratio and temperature) are being tested to determine whether it effect sugar recovery. It shows that all parameters are affect sugar recovery.

CHAPTER 1

INTRODUCTION

1.1 Background

Since the industrial revolution, the curiosity of human beings has driven us to explore new technologies and power to create a better civilization for human kind. From the ancient time to the creation of automobile by American industrialist, Henry Ford to the future Breakthrough Startshot interstellar spacecraft, petroleum is continually at the highest demand to be used in the new technologies especially in transportations and machineries. The threat of unavoidable global warming that gives negative impacts to environments together with declining crude oil reserves and soaring energy demands lead to the essential to replace fossil-based fuels with more environmental friendly biofuels.

Alternative energy or biomass energy is one of the sources that can replace crude oil to be used in advanced technologies. Although it has been extensively used as alternative energy, it also can be used in mining sector mainly in mineral processing. Biomass can be flourishingly used as a reductant to purify the impurities in mineral. Reductant typically used in metallurgy production of pig iron in steel industry where coke operates as a reducing agent but the excessive emission of CO_2 may cause climate change and other threats to Mother Earth.

Biomass-based fuels, such as bioethanol, provide a promising alternative, since their energy is already included in the global carbon cycle, implying, a significant reduction in carbon dioxide release (Karagöz *et al.*, 2012). Lignocellulosic biomass has been famously used as renewable sources to replace fuel and current reducing agent in metallurgical sector. Lignocellulosic is composed of carbohydrate polymers matrix containing cellulose and hemicelluloses but it covered with an aromatic polymer known as lignin. Malaysian elephant grass (*Pennisetum purpureum)* also known as Napier grass is one of a lignocellulosic material that has tremendous potential for carbohydrate polymers production in tropical countries like Malaysia due to their adaptability, availability and its non-food status as they do not compete with the food industry.

1.2 Problem Statement

Rising oil prices, climate changes and global energy crisis are the driven development of alternative energy. The uses of coke as reducing agent in steel industry also contribute to greenhouse effect because the emission of CO_2. To revolutionize the non-renewable energy for the remarkable future, Malaysian elephant grass is chosen to extract the carbohydrate polymers use to replace the non-renewable energy. The issue of extracting carbohydrate polymer also known as sugar is the lignin. Lignin is a type of complex organic polymers that form in most every plant. Besides that, lignin has rigid structures meaning it is not flexible and do not rot easily. To produce paper, cellulose-derives polymers and fuels require pure cellulose, so in the cellulose polymer industry, the presence of lignin in biomass is one of a major problem (López *et al.*, 2010). To improve the delignification process, more efficient and economically feasible elements need to be included into the delignification process.

1.3 Objectives

The objectives of this project using Malaysian elephant grass to recover sugar include:

1. To characterize Malaysian elephant grass physically before and after delignification and hydrolysis
2. To observe the effects of alkaline and acidic pretreatment efficiency
3. To determine how parameters effect the sugar recovery

1.4 Scope of Work

The scope of work in this study focus on characterization of Malaysian elephant grass before and after process of delignification and hydrolysis, to observe the effect of alkaline and acidic pretreatment efficiency and to determine how parameters effect the sugar recovery.

Malaysian elephant grass characteristics study was carried out to determine the physical characteristics before and after process of delignification and hydrolysis. Next, efficiency of alkaline and acidic pretreatment was carried out by determine the percentage of biomass recovery. Different parameters were used to determine the most efficient condition to delignify the Malaysian elephant grass.

1.5 Dissertation Outline

This dissertation contains five chapters:

Chapter 1 discusses more on the background, problem statement, objectives and scope of this study. It gives an overview for the study.

Chapter 2 explains the literature reviews about Malaysian elephant grass, their characteristics before and after delignification and hydrolysis, parameter examined to delignify the grass and ways to optimize the process of delignification.

Chapter 3 covers the methodology and technique used in this research. It includes taxonomy of the grass, sample preparation, the elephant grass characteristics study before and after pretreatments using SEM, and the process of the pretreatments.

Chapter 4 presents the results obtained from the experimental works. The results are presented in graphical form and analysis and discussion are made in detail,

Chapter 5 concludes the findings of the research and provide recommendations for improvement in future studies.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Most of the pollution of environment in Malaysia, the increasing of oil price and the decreasing of oil reserves worldwide which contributed to the uses of biomass energy as an alternative energy. In metallurgy sector, the uses of coke as a reducing agent in steel production also contributing the environment impacts due to excessive amount of CO_2. Demand for energy and preservation of the natural environment to meet economic and social development and improve human welfare and health are increasing.

Biofuels offer the imminent to lessen the dependence on utilization of petroleum derivatives, address the fuel security and condition issues, and support some financial advantages, for example, reasonable advancement and making occupations (Cai *et al.*, 2017). Expanding consideration has been paid on looking for inexhaustible and option vitality sources to tackle the present vitality and ecological issues emerging from the usage of fossil vitality (Li *et al.*, 2017). Among all the proposed options, biomass is considered as a standout amongst the most encouraging sustainable power sources because of its likeness with fossil in giving fuel and chemicals. Lignocellulosic biomass is the most bottomless and inexhaustible material on the planet to create biofuels. Utilizing lignocellulosic biomass determined biofuels could diminish dependence on petroleum derivatives and add to environmental change alleviation (Toklu, 2017).

However, the effectiveness of the uses of lignocellulosic biomass is mainly depends on the physical and chemical contains of the plants. Therefore, to extract sugar from the plant, the study of characterization, delignification and hydrolysis of the plant are important to extract the sugar economically without effecting the environment or plants that have food status that may affect the ecology systems.

2.2 Lignocellulosic Biomass

Conventional biomass is divided into two gatherings: lignocellulosic biomass and algal biomass. Lignocellulosic biomass contains three primary segments, to be specific cellulose, hemi-cellulose and lignin, while the algal biomass is predominantly made out of starches, proteins and lipids (Li *et al.*, 2017). Utilizing lignocellulosic biomass determined biofuels could diminish dependence on non-renewable energy sources and contribute to climate change mitigation. A significant comprehension of the physicochemical properties of lignocellulosic biomass and the logical portrayal techniques for those properties is basic for the design and operation of related biomass conversion processing facilities (Cai *et al.*, 2017). Bioenergy can be created from an assortment of biomass feed stocks, including forest, agricultural and domesticated animals deposits; short-rotation forest plantations; energy crops; organic component of municipal solid waste; and other organic waste streams (Toklu, 2017).

2.2.1 Elephant grass (*Pennisetum purpureum*)

Grass is the world's cheapest lignocellulosic biomass; people often treat grass species as weeds or feedstock for animals and have not realized other importance of grasses. However, grasses are capable of becoming a potential producer of lignocellulosic biomass (Liong *et al.*, 2012). Elephant grass (*Pennisetum purpureum*) is a lignocellulosic material that has high potential for ethanol generation in tropical nations because of their high accessibility and versatility. These points of interest permit a simple adjustment of various species and the advancement of vitality harvests, for example, grasses and forages. Among these, elephant grass (*Pennisetum purpureum*) has a high creation yield of dry material, i.e., 40–50 tons/sections of land/year, under ideal states of growth and management (Eliana *et al.*, 2014). Napier grass (*Pennisetum purpureum* Schum.) is a promising minimal effort crude material which does not compete with food prices, has appealing yields and an environmentally friendly farming (Camesasca *et al.*, 2015).

2.2.2 Components in Lignocellulosic Biomass

Lignocellulosic materials are unbending structures comprising of a starch polymer grid (for the most part cellulose and hemicelluloses). They are cross-connected and clearly bound to lignin. This structural complexity defined as biomass recalcitrance, severely restricts enzymatic and microbial openness (Toquero and Bolado, 2014). Lignin, the phenylpropane polymer (C9 units) exhibit in all plants, is one of the real parts of lignocellulosic biomass, for example, sugar cane, corn, rice straws, bamboo and "napier grass". Nonetheless, the presence of lignin in biomass is a noteworthy issue in the cellulose polymer industry. (De Araújo Morandim-Giannetti *et al.*, 2013). Lignocellulosic biomass is altogether different from biomass with vast substance of sugars or starch which is usually utilized as a part of biofuel industry. The structure of these former materials, made by cellulose, hemicellulose and lignin, requires the procedure for biofuels production to be adjusted for each type of biomass, according to their component characteristics (Eliana *et al.*, 2014). Cellulose fibers, nonetheless, are embedded arrange of lignin-hemicellulose. This system hinders cellulose biodegradation by cellulolytic enzymes (Esteghlalian *et al.*, 1997). It has four main sources: (1) agricultural residues (corn stalk, corn stover, sugarcane bagasse), (2) forestry residues (wood waste, sawdust, mill scrap), (3) energy-woody crops (willow, poplar, switch grass), and (4) industrial and municipal solid wastes (paper mill sludges, reused daily paper, wasted paper) (Wijaya *et al.*, 2014). The schematic diagram of goals of pretreatment on lignocellulosic material is shown in Figure 2.1.

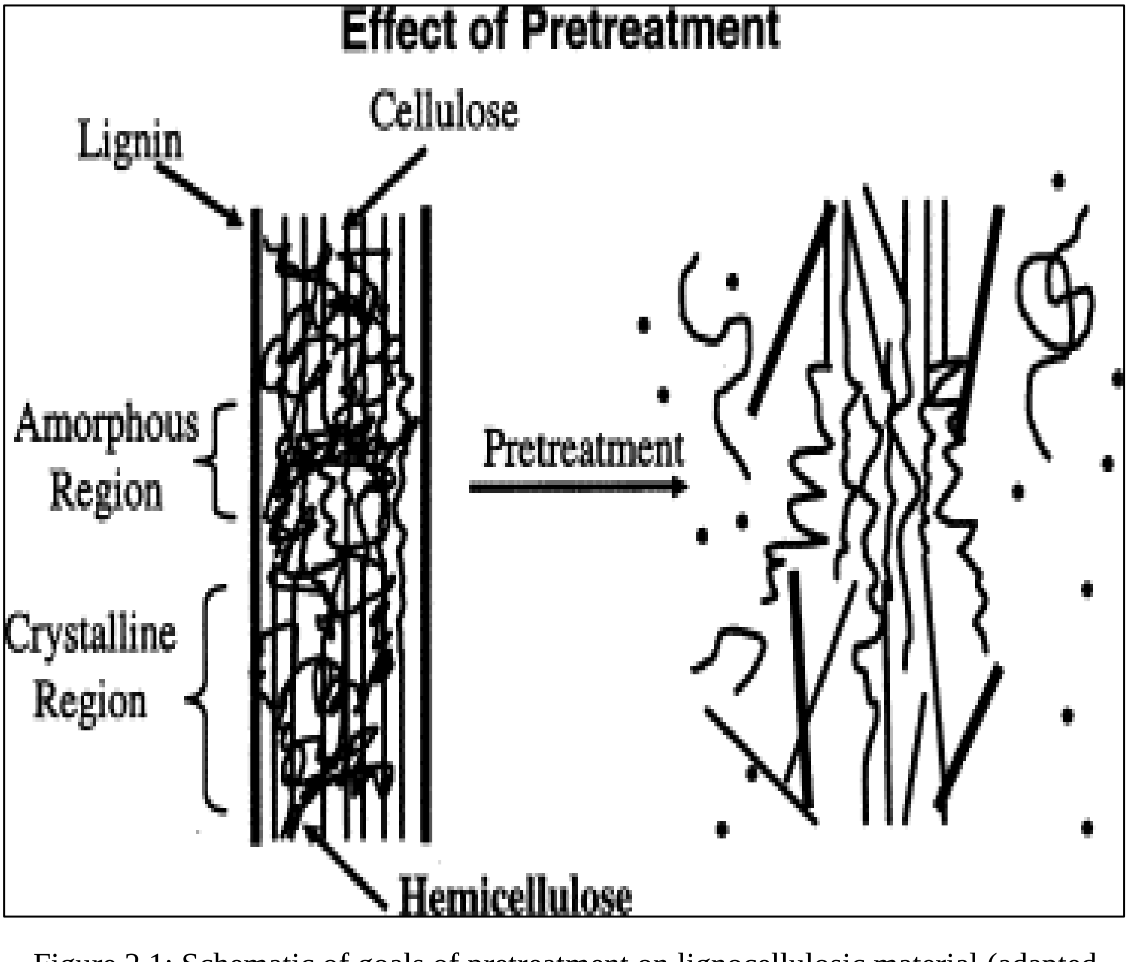

Figure 2.1: Schematic of goals of pretreatment on lignocellulosic material (adapted from Hsu et al., 1980)

2.3 Application of Lignocellulosic Biomass

2.3.1 Bioenergy

The decreasing supplies of petroleum, the production of cellulosic ethanol from biomass is viewed as a promising other option.(Pu *et al.*, 2013). Also, the utilization of lignocellulosic materials as fluid powers can help in lessening ozone harming substance outflows. (Menegol, Luisi Scholl, *et al.*, 2014). The tropical species *Pennisetum purpureum*, or elephant grass, is viewed as another option vitality trim anticipated that would give some country with abundant and sustainable lignocellulosic biomass for biofuel production (Menegol, Scholl, *et al.*, 2014). In this unique situation, biorefineries may speak to a suitable and maintainable contrasting option to substitute the requirement for oil, coal, petroleum gas and other non-sustainable power sources, as they can change over biomass into chemicals, energy and other fundamental material (Fontoura, Brandão and Gomes, 2015).

2.3.2 Reducing agents in metallurgy sector

Reducing agents used to oxidized metal to remove impurities to achieve more pure metal. In the 21st century, the applications of biomass have been used in the iron and steel metallurgical production, for the most part as fuels and reducing agents for various metallurgical processes. Compared with petroleum derivatives, low ash and high votality attributes for biomass offer many advantages in using biomass as a fuel or reducing agents in the ferrous metallurgical production (Wei *et al.*, 2017). The utilization of organic reductants to leach manganese dioxide metals has been appeared to be effective and straightforward. Be that as it may, there has been little commercial uses of this technique, due to the expense of the reductant and its high consumption rate in the process (Tian *et* *al.*, 2010). An intriguing characteristics for these biomasses are their sensible lower calorific esteem (LCV) and the capacity of being compacted into pellets or briquetted, which allows a significant increment on the lower calorific value and upgrades storage and transportation, and the amount of thermal energy that can be potentially recovered (Oliveira *et al.*, 2015).

2.3.3 Other applications

The novel properties of nanomaterials, for example, carbon nanotubes have made researchers and enterprises consider their application in certain fields. In particular, in medicinal services, nanotubes have developed as a noteworthy nanomedicine device giving huge advantages regarding drug targeting, delivery and release, and potential to combine diagnosis and therapy (Koutinas *et al.*, 2015). *Pennisetum purpureum* holds good possible for the efficient conversion of solar energy to biomass because, as a C4 plant, it possesses adaptations to suppress photorespiration (Menegol, Scholl, *et al.*, 2014). The natural fibers are likewise broadly utilized as a part of thermo plastics, for example, polyethylene, polypropylene, and polyvinyl chloride in the preparation of green composites (Reddy *et al.*, 2009). Cellulases have a few applications however to date have been basically utilized as a part of the material business for denim fading (Basso *et al.*, 2014). Pure cellulose for the production of paper, cellulose-derived polymers, and fuels (De Araújo Morandim-Giannetti *et al.*, 2013).

2.4 Extraction of sugar

2.4.1 Delignification process

Biomass pretreatment for depolymerizing lignocellulosics to fermentable sugars has been studied for nearly 200 years. Researches have aimed at high sugar production with minimal degradation to inhibitory compounds (Rabemanolontsoa and Saka, 2016). Different pretreatment varieties of alkali peroxide procedure especially with acids have been examined for lignin expulsion to improve hydrolysis (Bohórquez, Amado-gonzález and Martínez-reina, 2015). To make cellulose more accessible, pretreatment is required to alter the structure of cellulosic biomass. The enzymes will convert the carbohydrate polymers into fermentable sugars. A viable pretreatment is characterized by a few criteria. It avoids the need for reducing the size of biomass particles, preserves the pentose (hemicellulose) fractions, limits formation of degradation products that inhibit growth of fermentative microorganism, minimizes energy demands and limits cost (Mosier *et al.*, 2005). To determine their effect on the hydrolysis and the fermentability of the cellulosic fraction of this material, chemical and physicochemical pretreatments like alkaline peroxide, alkaline delignification, aqueous ammonia soaking, steam explosion, and diluted acid hydrolysis were performed. A scientific study of the effect of the alkaline pretreatment conditions (temperature, solid to liquid ratio, NaOH concentration and residence time) on the fermentability of elephant grass was carried out. Results showed that under pretreatment conditions of 120˚C for 1 h with 2 wt.%. NaOH solution and a solid to liquid ratio of 1: 20 (wt.) the highest yield of ethanol was collected, i.e., 26.1 g/L (141.5 mg ethanol/g dry biomass, 95% of theoretical yield). characterized before and after the pretreatments is a must to determine the contents of cellulose, hemicellulose, lignin,ash, extractives in the solid (Eliana *et al.*, 2014). To removes lignin from the biomass, alkaline pretreatment is one of the process. The removal of acetyl and various uronic acid substitutions on hemicellulose that against the enzyme to access the hemicellulose and cellulose surfaces (Minmunin, Limpitipanich and Promwungkwa, 2015). In addition to a low-cost enzyme complex, the function of pretreatment of lignocellulosic material is to separate the lignin–cellulose–hemicellulose complex. These pretreatment processes can be thermal, chemical, physical, and biological, or involve a combination of processes; the decision of pretreatment technique relies on upon the required separation level and the end use intended for the substrate (Menegol *et al.*, 2016). Alkaline pretreatment is one of method that use of NaOH has been extensively used on grasses (Jin *et al.*, 2015). (Eliana *et al.*, 2014) utilized five kinds of method to pretreat elephant grass in order to improve the hydrolysis and ethanol fermentability. The results showed that NaOH- heat yield the most elevated centralizations of decreasing sugars and ethanol.

However, for lignocellulosic biomass pre- treatment, NaOH was one most effective chemical but it still had few disservices, e.g., causing corrosion of the reactor and loss of fermentable sugars via chemical changes of sample. The primary principle of alkaline-heat was delignification. The enzymatic hydrolysis of cellulose in lignocellulosic feedstock is affected by lignin content, distribution, and its irreversible adsorption to cellulose (Jin *et al.*, 2015). The schematic diagram of pretreatment process is shown in Figure 2.2.

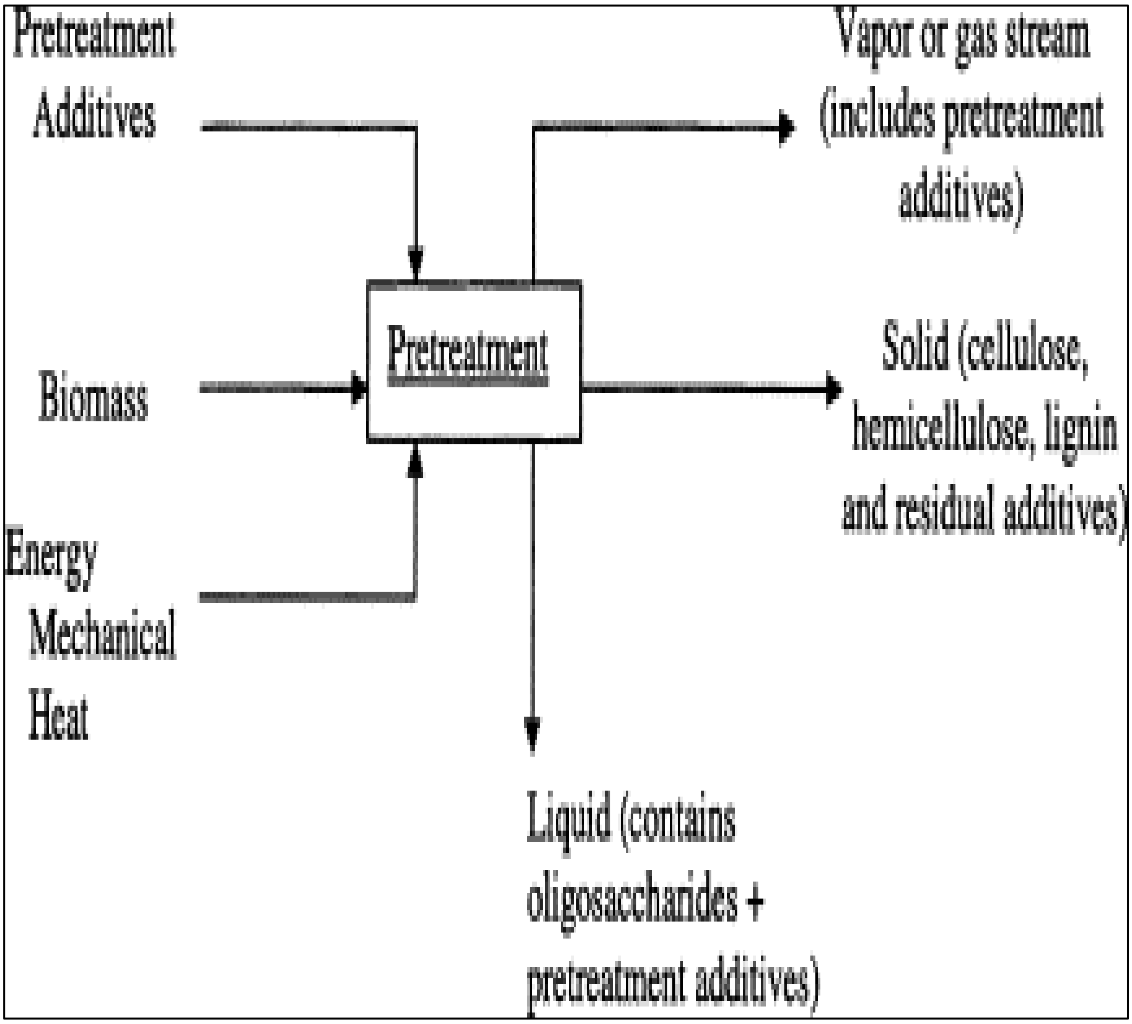

Figure 2.2: Schematic of Pretreatment Process (Mosier *et al.*, 2005)

2.4.2 Pre-treatments

2.4.2.1.Alkaline pretreatment

On account of the alkaline pretreatment, the changes in the composition are for the most part because of the removal of lignin and in a smaller proportion to the hydrolysis of cellulosic and hemicellulosic fractions, which leads to a decrease in the weight of the solid recovered after the pretreatment (Eliana *et al.*, 2014). Alkaline pretreatment technologies like NaOH, Ca(OH)2, NH3, and alkaline H2O2 (aH2O2), were utilized to delignify lignocellulose. The pretreatment step is crucial to lesssen the recalcitrance of plant biomass and plays a crucial role in biomass processing and biorefinery stages. (Phitsuwan, Sakka and Ratanakhanokchai, 2016). The great outcomes accomplished with the NaOH pretreatment are a consequence of the well known effects that this treatment has on lignocellulosic materials: (i) swelling of biomass, leading to an increase in internal surface area, (ii) changing the cellulose structure into a denser and more thermodynamically stable form, (iii) decreasing the degree of polymerization and crystallinity of cellulose, (iv) separation of structural linkages between lignin and carbohydrates and breaking-down of the lignin structure, and (vi) solubilization, condensation and redistribution of lignin (Eliana *et al.*, 2014). Pretreatment with NaOH solution disintegrates both cellulose and lignin contents. The best concentration of alkali pretreatment is 5.5 wt.% NaOH by soaking the grass at 70˚C for 2 hours (Minmunin, Limpitipanich and Promwungkwa, 2015). Contrasted with other chemical pretreatment technologies, alkaline hydrolysis can be done at lower temperature and pressure causing less sugar debasement than acid pretreatment yet the reaction times take several hours or days, or even weeks for softwood (Bali *et al.*, 2015).

Biomass pretreated with NaOH aqueous solution showed increment in porosity and a greater surface area and it can observe by scanning electron microscope, X-ray diffraction and Fourier transform infrared spectrometer. (Rabemanolontsoa and Saka, 2016). The alkaline pretreatments have been characterized by having a good capability of delignification without any significantly affecting the other components composition (Camesasca *et al.*, 2015). To remove residual sodium hydroxide, the insoluble residue was collected by vacuum filtration and washed with deionized water until neutral pH was reached (Chang *et al.*, 2011).

2.4.2.2.Acidic Pre-treatment

Acid hydrolysis of this grass diminished the hemicellulose content in the solid phase due to solubilization. It has been accounted that the acid hydrolysis of lignocellulosic materials solubilizes the hemicellulosic fraction by altering the covalent bonds, hydrogen bonds and van de Walls forces of this fraction (Eliana *et al.*, 2014). Same behaviour was evident when sulphuric acid was used in elephant grass pretreatment. A decline in biomass recovery from 48.21 ± 4.78 % (5 % H2SO4) to 42.20 ± 2.47 % (10 % H2SO4) was seen with an increasing acid concentration. (Menegol *et al.*, 2016). It can meddle with the accurate quantification of polysaccharide sugars, Klason lignin, and acid soluble lignin, which can be isolated and measured. Two stages of acidic pretreatment undergoes typically a strong sulfuric acid hydrolysis step at room temperature and then a dilute sulfuric acid hydrolysis step at high temperature to break down to their monomeric forms (Cai *et al.*, 2017). 39% of initial napiergrass and 47% of xylan are been removed by the diluted acid (Camesasca *et al.*, 2015). The effects of pretreatment conditions including acid concentration, pretreatment temperature, and reaction time on the disintegration of biomass components (cellulose, hemicelluloses, and lignin), sugar yield, formation of fermentation inhibitors, and enzymatic digestibility of the pretreated OPT will be investigated (Noparat *et al.*, 2015). Concentrated acid allows to acquire high yield of sugars, for example glucose from cellulose with low temperature. Hydrolysis rate is however, it lowers the crystalline cellulose than for amorphous hemicellulose due to their difference in intrinsic properties. When contrasted with concentrated acid, dilute acid hydrolysis presents the advantage of lower acid consumption yet consequently, higher temperature is required and strong conditions should be applied to achieve reasonable yield of glucose from crystalline cellulose, resulting in an extensive degradation of the amorphous hemicelluloses (Rabemanolontsoa and Saka, 2016).

2.5 Journal's Results

Based on the Figure 2.3, Figure 2.4, Figure 2.5 and Figure 2.6 show the pretreatment results by (Eliana *et al.*, 2014) and (Minmunin, Limpitipanich and Promwungkwa, 2015) and the results were compared with the current result. Table 2.1 shows the summary of samples and reagents used by other journals.

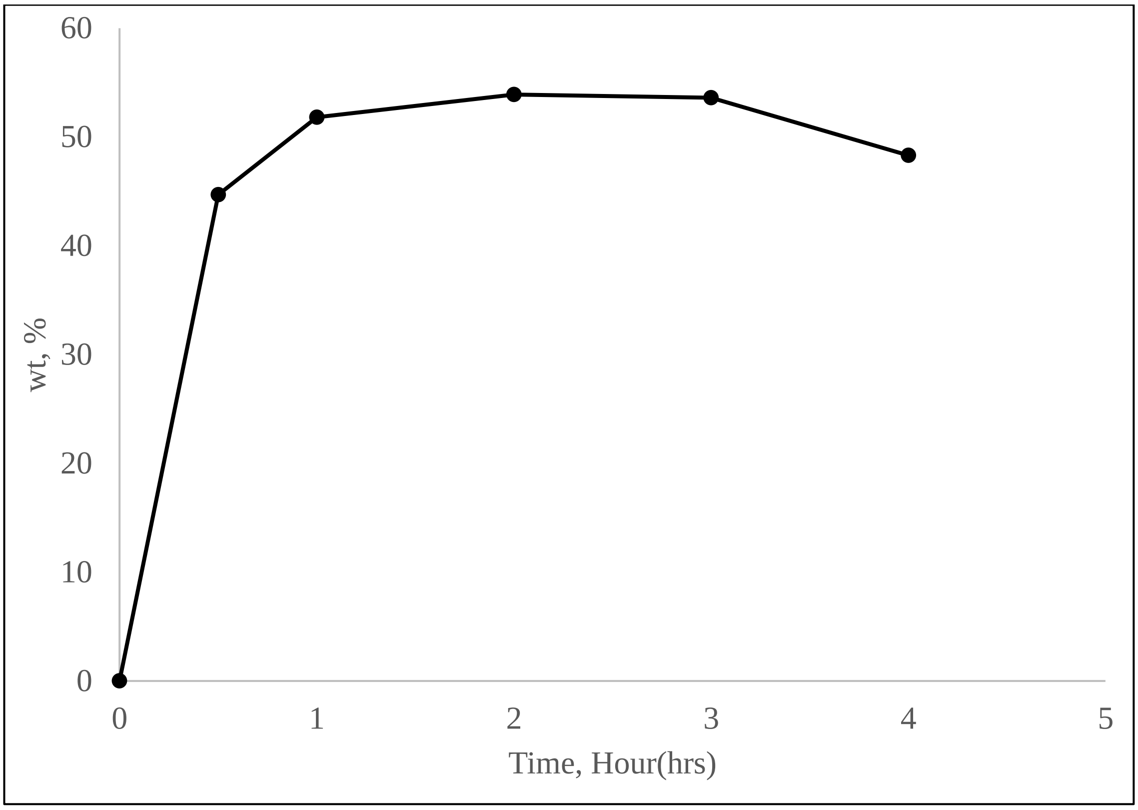

Figure 2.3: Alkaline pre-treatment, weight loss percentage using time as a parameter (Eliana *et al.*, 2014)

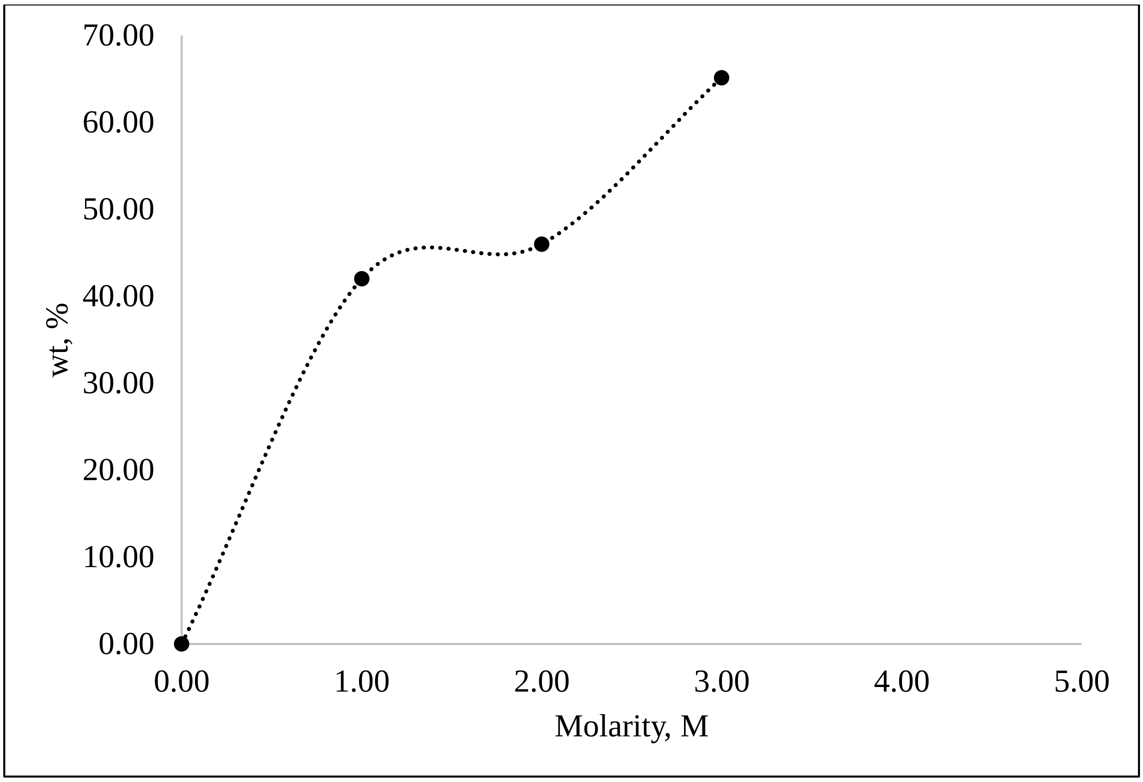

Figure 2.4: Different concentration of NaOH solution (Minmunin, Limpitipanich and Promwungkwa, 2015)

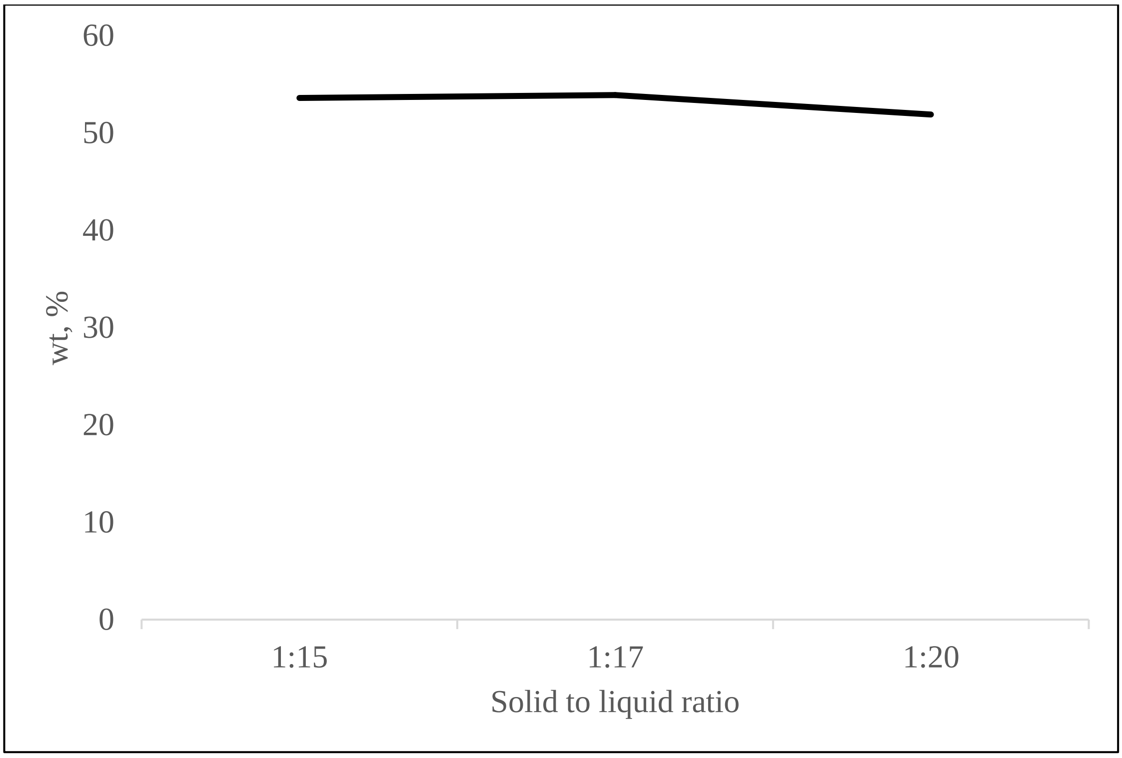

Figure 2.5: Solid to liquid ratio, to optimize alkaline pretreatment (Eliana *et al.*, 2014)

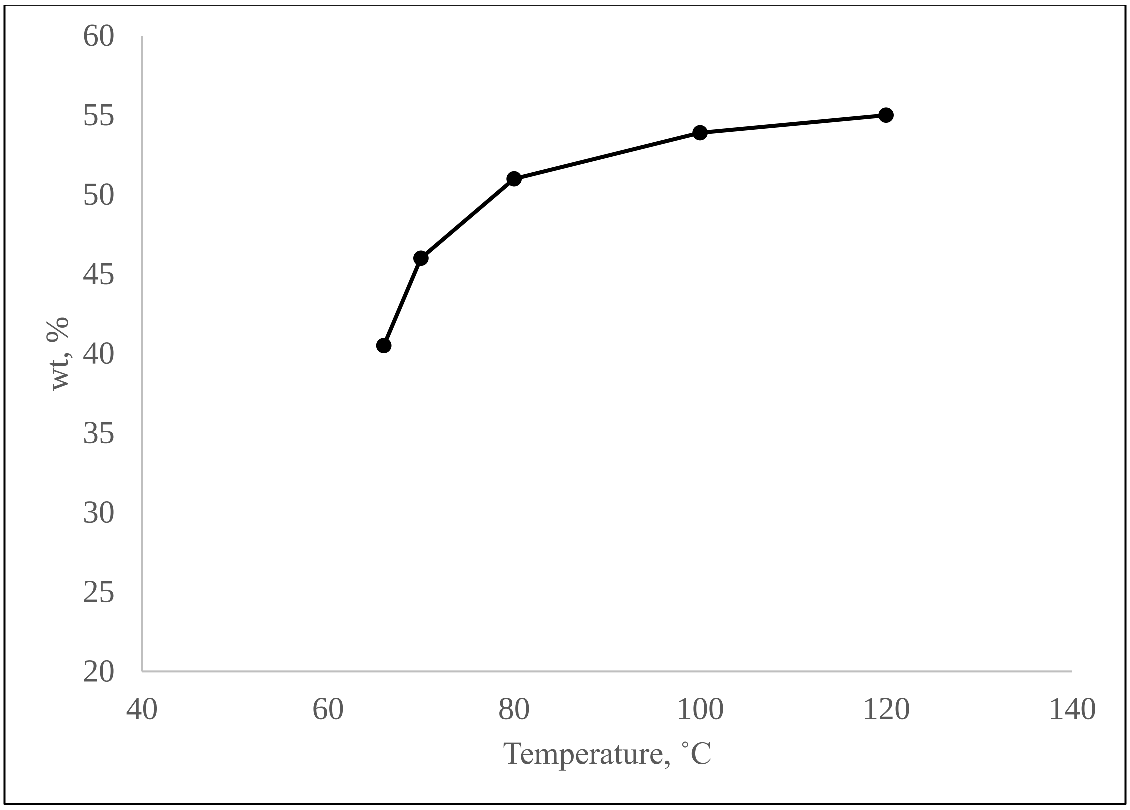

Figure 2.6: Alkaline pretreatment under different temperatures (Eliana *et al.*, 2014) and (Minmunin, Limpitipanich and Promwungkwa, 2015)

Table 2.1: Summary of samples and reagents used by other journals

Sample	Reagent	Best result	Writer
Napier grass	NaOH	53.9%	(Eliana *et al.*, 2014)
	NaOH	46.32%	(Minmunin, Limpitipanich and Promwungkwa, 2015)
	NaOH	51.33%	(Menegol *et al.*, 2016)
	H_2SO_4	48.93%	
	NH_4OH	65.43%	
	NaOH	52.44%	(Phitsuwan, Sakka and Ratanakhanokchai, 2016)
	$CaOH_2$	67.51%	
	NH_3	65.63%	
	CH_3COOH	67.45%	(Takata *et al.*, 2013)
	Phosphoric acid	64.56%	

2.6 Summary

Based on the literature review, it can be concluded that the pretreatment and delignification process are crucial for the recovery of sugar. The lignin is a major problem to extract sugar because lignin has a rigid structure and it is difficult to broke in physical ways. Since the features of lignin prove to make the crops quite challenging to process, materials with low lignin and high cellulose and hemicellulose contents are more desired for future biomass processing and production (Jung et al. 2015). Although, lignin is difficult to remove, extensive researches and modifications have been carried out to optimize pretreatment and delignification process. Therefore, this study is carried out to observe the effect of alkaline and acidic pretreatment efficiency and to determine the best condition to delignify the Malaysian elephant grass.

CHAPTER 3

METHODOLOGY

3.1 Overview

Laboratory works were conducted in this study to investigate the characteristics Malaysian elephant grass physically before and after delignification and hydrolysis, to observe the effect of alkaline and acidic pretreatment efficiency and to determine the best condition to delignify the Malaysia elephant grass. The Malaysian elephant grass is collected at Penanti, Bukit Mertajam, Pulau Pinang was first characterized using SEM. Specimen voucher containing the taxonomy of the grass determined by herbarium process.

The process of delignification was started with sample preparation where the grass was cut at certain size. The cut grass was dried at certain temperature and continued with laboratory tests.

After the laboratory tests, the performance of delignification of the selected parameters were determined by calculating the percentage of biomass recovery. The test was conducted to determine the best condition to delignify the Malaysian elephant grass and which pretreatment has highest efficiency the remove lignin. SEM was used to determine the surface morphology of the grass after process of delignification. The overall methodology of the research is summarized in Figure 3.1.

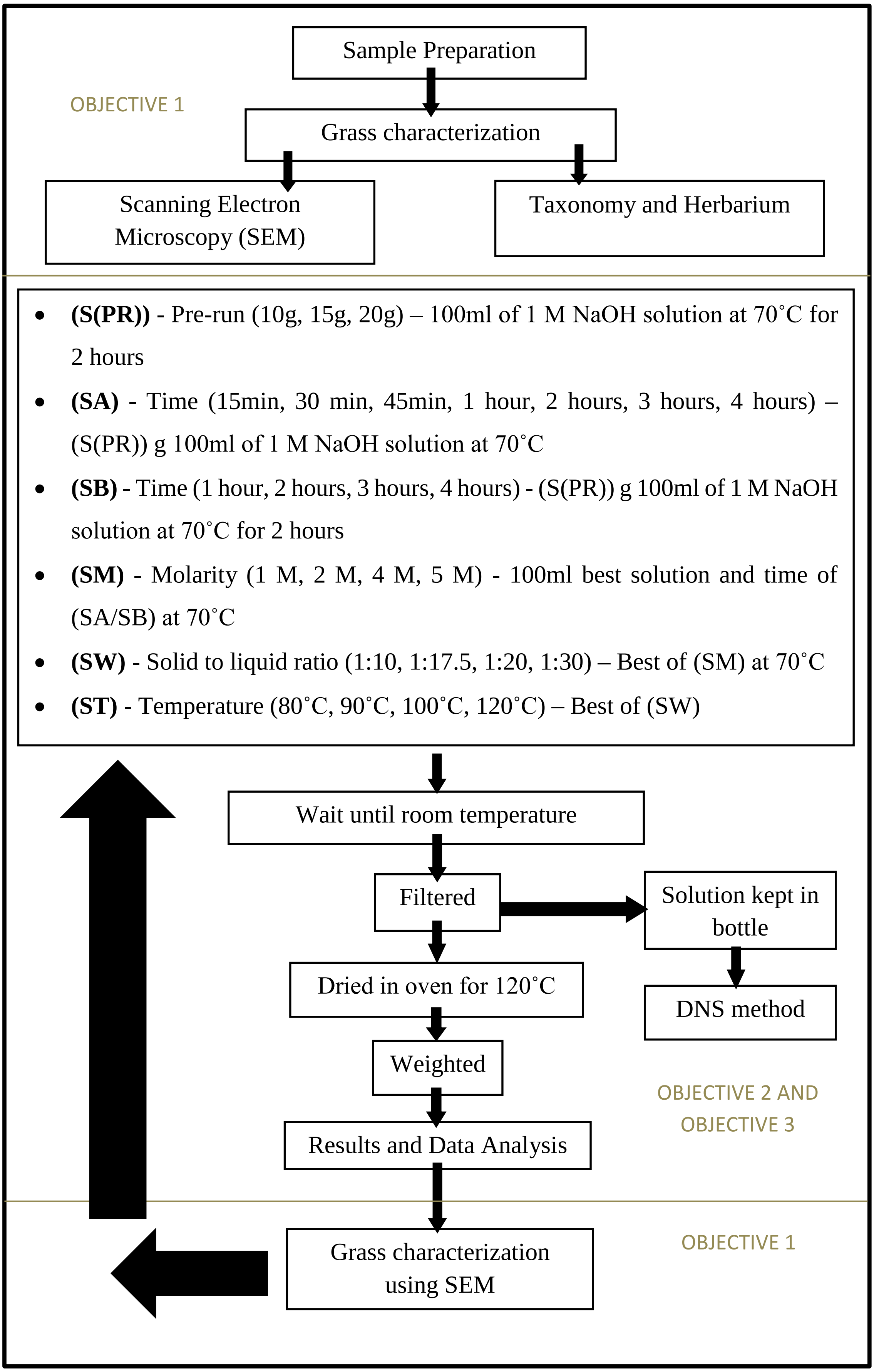

Figure 3.1 : Flow Chart of Methodology

3.2 List of chemicals and equipment used

3.2.1 Chemicals

The preparation, usage, storage, disposal were followed as regulated in the Material Safety Data Sheet (MSDS). The chemicals used in this experiment are shown in Table 3.1.

Table 3.1: List of chemicals used in delignification process

Chemicals	Formula	Molarity, M
Sodium Hydroxide	NaOH	1, 2, 4 and 5
Sulphuric Acid	H_2SO_4	1, 2, 4 and 5

3.3.2 Apparatus

The apparatus used in the experiments was shown in Table 3.2. All glassware is washed before being used to prevent contamination that may affect the result.

Table 3.2: List of apparatus used in this experiment

Glassware/Apparatus	Unit	Description
Beaker	2	500 ml
Conical Flask	2	500 ml
Thermometer	1	-
Glass Rod	1	-
Tea Filter	1	-
Oil Bath	1	With stands and clamp

3.3 Sample Preparation

The Malaysian elephant grass was taken at Penanti, Bukit Mertajam, Pulau Pinang were cut. The stem is separated with the leaves. The mid ribs from the leaves are been removed. Then, the leaves were cut with 1 cm x 1 cm size as shown in Figure 3.2. After the process of size reduction process, the grass was oven dried (115˚C) for 3 hours to remove all the moisture. Lastly, the grass was stored in air tight container for later use.

Figure 3.2: Size reduction 1 cm x 1 cm

3.4 Characteristics Study

Physical characterization of the grass was carried out using SEM instrument. This test was carried out for both before and after the process of delignification. Process of defining the genus and the species and specimen voucher of the grass defined by herbarium process.

3.4.1 Taxonomy and Herbarium

Taxonomy is the process of classifying the genus and species of the grass based on their physical characteristics also known as herbarium where the collected plants have been dried, pressed, preserved and classified. The Malaysian elephant grass has been sent to School of Biological Sciences from Universiti Sains Malaysia. The methods of herbarium were:

1. A complete part of the grass including flowers, stems, leaves and root system were collected.
2. The grass is washed and the washed grass was spread out between the folds of old newspapers to avoid parts overlapping. The grass is dried for 24 to 48 hours.
3. The grass was preserved with Formalin Acetic Alcohol for weeks
4. The preserved grass then mounted on herbarium cupboard of standard size (41 x 29cm) in their proper position. It can be done by sewing the grass with the herbarium cupboard.
5. The label containing information about the locality, date and time of collection, name of collector, common name, complete scientific name and habit are printed and pasted on the lower right hand corner.
6. The taxonomy and specimen voucher determined and given by plant specialist.