**SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING**

**UNIVERSITI SAINS MALAYSIA**

# EFFECTS OF HYDROLYSIS USING VARIOUS PARAMETER FOR RECOVERY OF REDUCING SUGAR FROM LOCAL COCONUT DREGS

By

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## DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled “Effects of Hydrolysis Using Various Parameter for Recovery of Reducing Sugar from Local Coconut Dregs”. I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or University.

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SYMBOLS

| | |
|---|---|
| ˚C | **D**egree **C**elsius |
| % | **P**ercentage |
| g | **G**ram |
| ml | **M**illiliter |
| 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 |
| DNS | Dinitrosalycylic Acid |

KESAN PENGGUNAAN BEBERAPA PARAMETER DALAM HIDROLISIS HAMPAS KELAPA TEMPATAN UNTUK MENGEKSTRAK GULA PENURUN.


## ABSTRAK

Produk selepas hasil perahan santan iaitu hampas kelapa adalah salah satu daripada sumber penghasilan bahan bakar semulajadi yang mampu mengurangkan masalah persekitaran. Kandungan karbohidrat seperti selulosa dan separa-selulosa di dalam hampas kelapa boleh dihidrolisis untuk menghasilkan produk seperti bio-etanol and ejen penurunan bagi penggunaan industri metalurgi. Objektif kajian adalah bertujuan untuk menyiasat dan mengkaji hidrolisasi hampas kelapa. Yang pertama, ciri fizikal dikaji untuk mengetahui perubahan pada permukaan hampas kelapa sebelum dan selepas proses hidrolisis pembuangann lignin dan hidrolisis tidak langsung menggunakan asid. Kemudian, hidrolisis pra-pemulihan dijalankan dengan membezakan penggunan larutan asid dan alkali, dimana larutan asid dipilih sebagai larutan pra-pemulihan pada kondisi meggunakan 6gram hampas kelapa dimasukkan ke dalam 100mL asid sulfuric mengandungi kepekatan 0.5% pada suhu 70˚C selama 2 jam, dimana ia memberi kehilangan berat sebanyak 9.33% tanpa menghidrolisiskan glokosa. Seterusnya, hasil pengeluaran yang terbaik dari hidrolisis secara langsung dan tidak langsung menunjukkan hidrolisis tidak langsung menghasilkan lebih tinggi kehilangan berat dan pengekstrakan glukosa dengan pertama melakukan hidrolisis pra-pemulihan dan bersambung pada kondisi terbaik dalam parameter keseluruhan dimana, sampel 6gram hampas dari pra-pemulihan dimasukkan ke dalam 100mL asid berkandungan 3% kepekatan pada suhu 90˚C selama 2 jam, jumlah 4 jam. Kandungan glukosa ditentukan dengan mengguna cara kalorimetri DNS dan analisis dilakukan dengan meggunakan alat UV-Visible Spektrometri dan akhirnya kandungan glukosa tertinggi diperolehi adalah 1.263g/L dengan menggunakan kondisi terbaik dalam hidrolisis tidak langsung.

EFFECTS OF HYDROLYSIS USING VARIOUS PARAMETER FOR RECOVERY OF REDUCING SUGAR FROM LOCAL COCONUT DREGS

## ABSTRACT

The coconut dregs one of the source to create alternative fossil fuel that could help reduce a lot of environmental problems. The content of carbohydrates of cellulose and hemicellulose inside coconut dregs can be hydrolyzed to produce bioethanol and also reducing agent for metallurgical industries. The aim of this study is to understand and investigate the hydrolysis of coconut dregs. First, physical characterization study was done to study their alteration on surface morphology after best of delignification and all indirect acid hydrolysis was done. Next, the pretreatment of coconut dregs was done by comparing acid and alkali pretreatment and acid solution was selected as the best pre-treatment solution at condition of using 6g of coconut dregs immersed in 100mL of 0.5% of $H_2SO_4$ solution at 70˚C for 2 hours, which gives 9.33% weight loss and 0% glucose yield. Next, the best of indirect and direct hydrolysis outcome shows that indirect acid hydrolysis has higher weight loss and glucose yield compared to direct acid hydrolysis. And the final conditions that is yielding highest weight loss and glucose yield is by first doing pre-treatment as in best of pre-treatment condition and continued process to the best of all parameter with conditions of, 6g of coconut dregs after pre-treated immersed in 100mL of 3% concentration fresh acid solution at 90˚C for 2 hours and have total of 4 hours for indirect acid hydrolysis. The Glucose content was determined by using DNS calorimetry method analyzed using UV-Visible spectrometry and give the highest yield of 1.263g/L with best of indirect acid hydrolysis condition.

CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

In the present, with the world population that keep increasing together with rises of modern technologies has brought demands that could bring devastation to the earth's environment. The risk of global warming that cause climate changes also lead the purpose of creating the biofuel with present technology (Hasan et al., 2016). Will human be able to live without any progression towards the cleaner way of handling technologies that is keep growing rapidly? Due to massive consumption of fossil fuel in various industry throughout the world and still increase in demand, the society ought to find alternative methods in finding fossil fuel replacements to avoid depletion of non-renewable source when its time of need.

To reduce the harmful done to environment of the earth, even an organization like European Union are taking the first step in the effort that enforcing scale down the uses of environmental deteriorating materials such as fossil fuels (Isikgor and Becer, 2015). Presently, starch is the major feedstock in converting fermentable sugar to biofuel or known as bioethanol (Jin, 2017). Various lignocelluloses materials like agrarian waste, woodland deposits, biomass waste, waste paper and metropolitan solid wastes are predominantly comprised of carbohydrates and they also comprised of extractives and ash (Bujang et al., 2013).Lignocellulosic biomass waste is rather cheap, high quantity and easily obtained from agro-industries will be the substantial reduced cost and eco-friendly (Anwar et al., 2014).

Certain lignocellulosic material could also be applied in quarry industry as the additive in the Portland cement (Roopan and Elango, 2015). The study on this project research is specifically on recovery of reducing sugar from coconut dregs. These reducing sugar came from the major compound of carbohydrates in which are cellulose and hemicellulose. In Malaysia, coconut is infamous for its use in traditional food and also health cure. Coconut dregs is the waste byproduct of coconut milk extraction where it is usually will be dump or either will be made as animal's feedstock. Thus, this thesis writing will be discussing the use of coconut dregs as a preparation substance in the making of biofuels.

## 1.2 Problem Statement

The researches on extracting reducing sugar from lignocellulosic biomass has been done quite extensively. There were several types of species of plants and woods that have been studied of their content of lignin, hemicellulose, cellulose and some other compositions. However, there is still lack of study in this research of utilizing coconut dregs on recovering reducing sugar (Bujang et al., 2013).

Till this day, the way to degrade cellulose to glucose are either by chemical hydrolysis or enzymatic hydrolysis (Wang, 2017). The study from (Woiciechowski, 2002) ,enzymatic hydrolysis shows higher cost process since economical production enzyme use slightly higher energy consumption than acid which specifically Sulfuric acid for this project. Cost-effective, efficient conversion of plant cell walls into their components, mainly carbohydrates and lignin, is fundamental to realizing the full potential of the biomass lignocellulose feedstock. Lignin is particularly hard to degrade and represents the major barrier to efficient utilization of the plant biomass. Consequently, some mechanical, chemical, microbial or enzymatic pretreatments for lignin modification and/or removal are

required, being their efficiency highly dependent of the lignin structure. For this reason, the knowledge of the exact structure and composition of the lignin polymer is important to find appropriated pretreatment and cellulose degradation methods (Rencoret et al., 2013).

Then, the last problem that to be state is the handling of the raw samples, storage of dried samples and hydrolysate of reducing sugar from coconut dregs hydrolysis to come. As known, materials contain sugar if let exposed will be rotten unless specific conditions are taken measure.

### 1.3 Objectives

The aims of the study are:

1. To conduct physical characterization for coconut dregs before and after both process of delignification and concentrated acid hydrolysis.

2. To study the effective delignification process between alkali and acid-based hydrolysis.

3. To study the effectiveness of several parameter between the direct and indirect acid hydrolysis in degrading cellulose and hemicelluloses in reducing sugar recovery.

4. To determine the quantitative reducing sugar obtained by DNS method.

## 1.4 Scope of Study

This study will be carried out mostly in experimental works. For the first work is, characterization study using SEM to cover the first objective. The purposes will be to observe the surface morphology of raw materials and the effects on coconut dregs after hydrolysis that will be done. Next, for second objective to determine the best delignify solution between alkali and acid. Two parameters will be run this objective and they are amount of sample and hydrolysis time. Also, this study is also important to understand the structural degrade of major component of lignocellulosic biomass for both delignification, Indirect and direct acid hydrolysis process. Lastly, the analysis of UV-Visible Spectrometry to determine the reducing sugar (glucose) content inside coconut dregs hydrolysate. This will cover the final objective of the project.

## 1.5 Thesis Outline

There are five chapters exist in this thesis which are introduction, literature review, method, result and discussion and lastly the conclusion and recommendation for future researches.

First chapter is generally the overview of the project that included the background of the study, problem statement, objectives, and scope of the project's study. Second chapter will basically explain on the general knowledge of the project's sample, previous works and information on reducing sugar extraction, and several processes of experimental work.

In third chapter, method of the experiment is thoroughly explained. It is included the overview of the experiment flow, lists of chemicals and equipment uses, characterization of samples using SEM, hydrolysis process and category, and also the analysis of sugar determination using UV-vis spectrometry.

Fourth chapter is the review of the results data after the experimental work is done and then will be properly discuss the outcomes based on the previous studies from literature review. And for the last chapter five, will be concluding the outcome from the results of the experiments done in this research. Recommendation will be point out of suggestion to improve the extraction of the reducing sugar from lignocellulosic biomass project in the future.

CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

### 2.1.1 Lignocellulosic Biomass

Lignocellulosic biomass is something that exist naturally everywhere in this world. One of major compound in these biomasses is carbohydrates. It also has been looked up as alternative resource of chemical feedstock that is potentially reduce the negative effects on the environment in the future (Kumar et al., 2009; Muensri et al., 2011). Several sources of lignocellulosic materials like agrarian waste, woodland deposits, biomass waste, waste paper and metropolitan solid wastes are comprised of carbohydrates and they also comprised of extractives and ash (Normah Bujang et al.). Recently, there were many organization that has interest in making these never-ending sources of lignocellulosic biomass as biofuels. The carbohydrates polymer is in form of lignin, hemicellulose and cellulose (Bujang et al., 2013). These are the components in the structure of sugar that will be needed for reducing sugar extraction.

This biomass also acts as a kind of solar energy because it is produced through photosynthesis. By absorbing sunlight, biomass is effectively able to store solar energy in significant quantities. As a result, biomass energy is a kind of solar energy, stored as chemical energy. Plants can convert carbon dioxide and water to glucose with the help of chlorophyll by photosynthesis and store the energy. Synthesized glucose can also be converted to compounds constituting plant body such as starch, cellulose, hemicellulose and lignin (Li, 2014). Moreover, uses of this energy will considerably lower global warming effects caused by greenhouse gas like carbon dioxide releasing from the use of fossil fuel. The use of biomass energy will not increase the amount of carbon dioxide in atmosphere

instead the plants will absorb them. Biomass energy is thus carbon neutral and cleaner energy than fossil energy (Isikgor and Becer, 2015; Li, 2014; Virgínio e Silva et al., 2018).

It is hard to make use of this substrate to produce the desired product just by using microorganism because of its natural structure. That is why hydrolysis process is significant in the conversion of complex structure lignocellulosic biomasses into fermentable sugar and cause the change in the characteristics of this substances. The use of dilute acid for biomass hydrolysis will degrade the hemicellulose into its sugar form was proven to give high reaction rates while also escalate the cellulose hydrolysis in high temperature and pressure condition. Mostly, dilute acid sulfuric acid is used in hydrolysis to remove hemicellulose and then boost the digestibility of the cellulose in the substrate. (Bujang, 2013)

### 2.1.2 Coconut palm (Cocos Nucifera)

Cocos nucifera is a scientific name for coconut palm which are in family species of Arecaceae. Cocos nucifera has various application and versatile in many field such as food, medical, biotechnology etc. It is mostly inhabiting in Asia pacific region and known as the king of tropical (Roopan and Elango, 2015). They have two distinct group which are tall and dwarfs (DebMandal and Mandal, 2011). Also, one of the most important sources of vegetable oil and found throughout the tropics. During Vasco da Gama's expedition, Portuguese introduced the coconut to Atlantic region about in 1498 and 1500' and they were brought from Indian ocean, they also brought in coconut palms along the West African coast, on the coastline of Brazil and on the Cape Verde archipelago. Coconuts had been introduced to the West Indies by 1582. Commercial planting of coconut palms began in the mid-l800's and was linked to the abolition of slavery in the British Empire in 1835

(Education, 2014). Figure 2.1 shows the typical type of coconut tree palm from arecaceae family breed in Malaysia.

Figure 2:1 The image of typical coconut palm tree from arecaceae family breed.

Figure 2.2, it shows the parts there is in a mature coconut. Starting from outer layer are the hard skin (exocarp) and inside it is husk or fibrous coir (mesocarp). Then, endocarp is the hard shell that cover the nutritious part of coconut. Innermost, it has two distinct endosperms, one in a liquid form called nut water and the other coconut meat is kernel. A matured kernel is utilized during the germination and early development of the embryo where it consists of nutrients like carbohydrate, fat, proteins, fiber, and minerals. During the process of germination, the embryo grows in two directions either the plumule or the shoot, grows towards the soft eye of the shell and the basal part of embryo develop into an absorbent spongy growth known as haustorium. Haustorium enlarges and fills the entire

water cavity in 20 to 24 weeks after germination (Manivannan et al., 2018). Since the study of this project is about coconut dregs, it will be more focused towards kernel part.

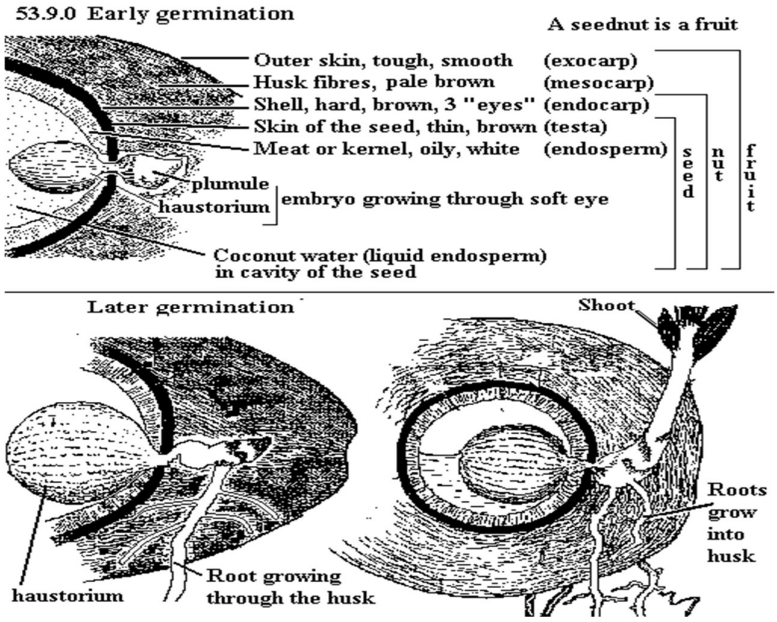


Figure 2:2: The image shows the components of coconut adapted from google image under (Education, 2014).

### 2.1.3 Application of lignocellulosic biomass

#### 2.1.3.1 Biofuel/Bioethanol

Until this day, fossil fuels has been the main feedstock in the production of liquid fuels for the uses of global vehicles, also chemicals and manufacturing industries. Carbon-neutral alternative can possibly be accomplished with the production of biofuel produced from biomass, where carbon released on combustion is balanced by its photosynthetic capture and replace the petroleum-derived fuels that has carbon complex (Marriott et al., 2015).


Figure 2:3: Bio-diesel from Caltex petrol station has been widely used in Malaysia.

In these past several decades, these has been a lot of researches done on renewable sources of liquid fuels to replace fossil fuels due to long-term economic and environmental concerns. Fossil fuels consumption such as coal and oil releases greenhouse gas like carbon dioxide, which is a major cause of global warming. One of the most practical way for improving energy security and reducing greenhouse emissions is by conversion of abundant lignocellulosic biomass to biofuels as transportation fuels and industrial fuel consumption (Kumar et al., 2009). Figure 2.3 shows the image of bio-diesel pump that exist in Malaysia's market.

### 2.1.3.2 Biocoke/Bio-reducer

To advance in the improvement of the environmental impact of industry, An idea of production of reducing agents like (biocokes or bio-reducers) by pyrolysis of biomass

is investigated and done(Adrados et al., 2016). The determination in production of biomass-derived coke is that the certainty of the processes involved neutral CO2 emissions (Isikgor and Becer, 2015; Virgínio e Silva et al., 2018). Biomass absorbs atmospheric CO2 while it grows and returns it into the atmosphere when it is burned, all in a short amount of time. Because of this, closed loop carbon cycle was created by biomass-derived product. Environmental-friendly biocokes is of the most promising reserve to replace the fossil fuel coke. The production of biocoke from biomass is not certainly unheard of, in fact it is the follow up of the conventional process of obtaining charcoal from wood.(Adrados et al., 2016).

#### 2.1.3.3 Other application

Nano-lignin has been an interest research as bio-based and bio-active nanomaterial fillers for use in bio-degradable composites. In its natural form, lignin is used as filler to improve the resistance of natural rubber vulcanizates to thermo-oxidative degradation in air (Juikar and Vigneshwaran, 2017). These day, there is an essential need for development of clean and non-toxic methods for nanoparticles synthesis as catalyst in organic reactions and medicinal field applications. That is the reason why many analysts are fixate concerning the environment-friendly ways of creating nanoparticles. Cocos Nucifera is one of the many plants that has been make use of by researchers.

The coconut crops' waste like husk and coir fibers was used as an addictive in Portland cement (Roopan and Elango, 2015). Even lignin has the potential use in industries to be which produce such as a dispersant reagent in cement and gypsum blends, absorbent agent, emulsifier and as a chelating agent (Welker et al., 2015).

## 2.2 Important Chemical composition of lignocellulosic biomass

### 2.2.1 Lignin

Lignin is the most complex and smallest fraction, and amount of lignin is different in various plants. Particularly, woods have lignin content that is ranged from 20 to 40% by weight. Lignin's main function in plants are to convey structural support of the woods and for water, nutrients and metabolites transport to entire body of plant (Harmsen et al., 2010; Li, 2014). It is an amorphous, polyphenolic material made up from settling of enzyme dehydrogenative polymerization of three phenylpropanoid monomers, Conniferyl, Synapyl and *p*-Coumaryl alcohols (Isikgor and Becer, 2015). It is also a heterogeneous polymer mainly composed of phenyl-propane units most commonly bind together by ether bonds in a long chain. Lignin mean to stick by layering the openings between and around the cellulose and hemicellulose complexion with the polymers. It is present in all plant biomass, so it is considered byproduct or as a residue in bio-ethanol production process (Anwar et al., 2014).

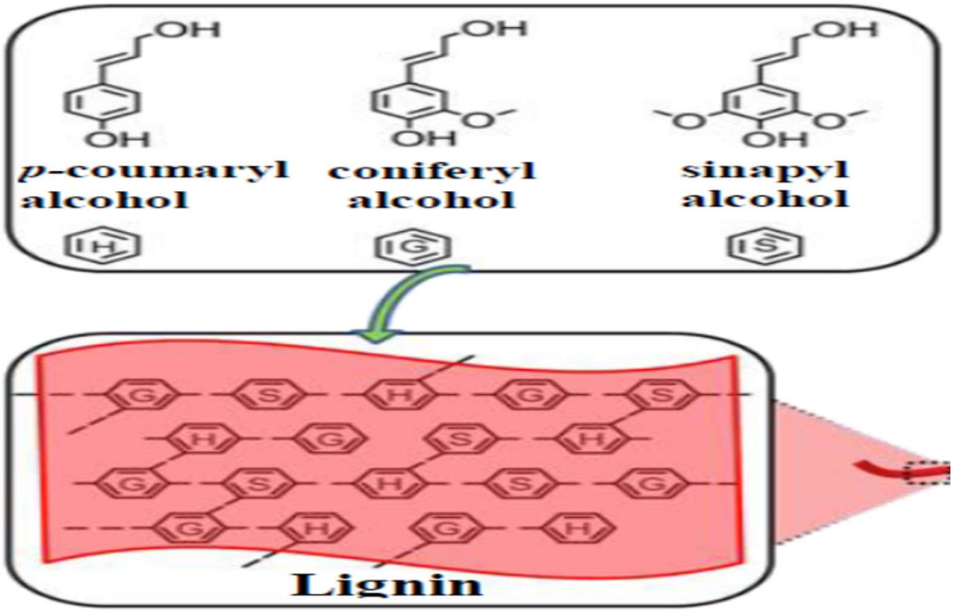


Figure 2:4: Structure of 3 types of phenyl-propane in Lignin adapted from (Isikgor and Becer, 2015).

### 2.2.2 Cellulose

Cellulose is a major component of reducing sugar for bioethanol production. It made up in great amount of glucose that could go through fermentation process to produce ethanol after being degraded. But, the crystallinity of cellulose makes enzyme hydrolysis become impenetrable. It is bind with D-glucose monomers to each other by linear β-(1,4)-glycosidic bonds which are quite solid, insoluble polymers of glucose that has shortage of side chains. Sequential glucose residues in the glucans are in repeating units and mirror look alike since it is arranged upside down symmetrically like in Figure 2.5. Many glucans chain formed in parallel crystalline arrays to form cellulose microfibrils, with the glucan chains held together by hydrogen bonds and van der Waals forces (Marriott et al., 2015).

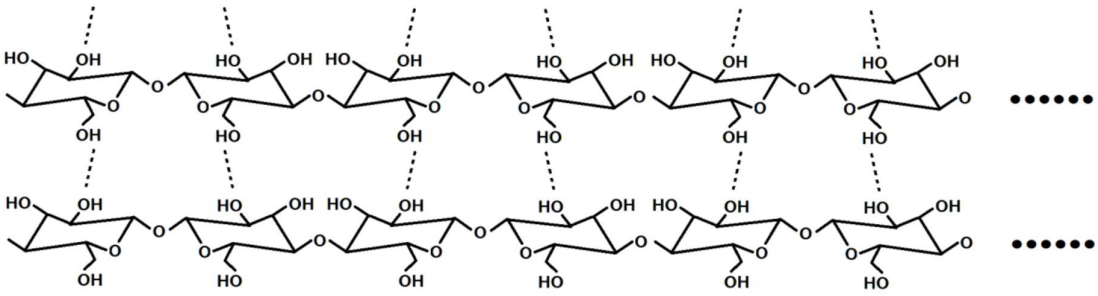

Figure 2:5: The structure of cellulose adapted from (Li, 2014)

### 2.2.3 Hemicellulose

Hemicellulose is the second most abundant heterogeneous polymers (Anwar et al., 2014). Hemicelluloses consist of various type of sugar monomers composed of Pentose such as L-arabinose, D-xylose and, Hexose such as D-galactose, D-mannose and D-glucose. It is not in solid formed or crystalline structure like cellulose (Anwar et al., 2014; Bujang, 2013). But rather, they form long chains that can branched together with cellulose by hydrogen bond which is easier to degrade into its subunits sugar form (Marriott et al.,

2015). Figure 2.6 shows the linkage of hemicellulose subunits sugar's structure. Table 2.1 shows the contents of lignin, hemicellulose and cellulose on several of the lignocellulosic biomass that has been researched before.

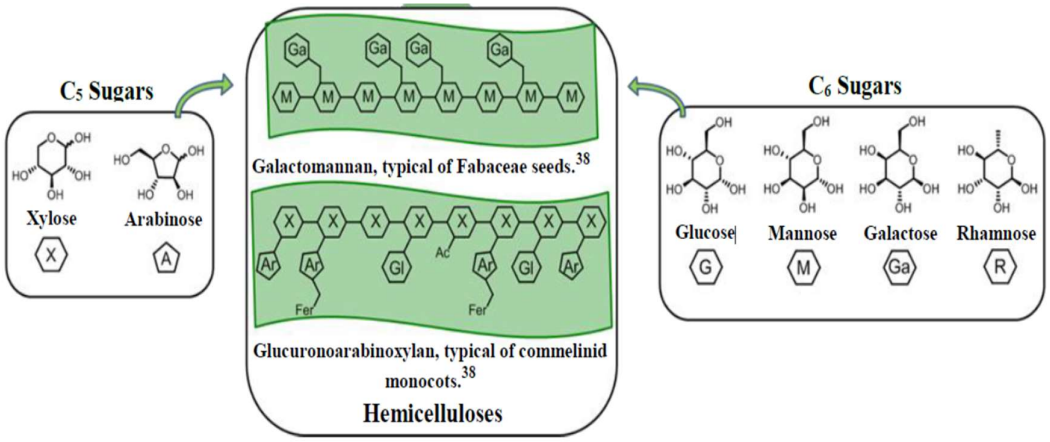


Figure 2:6: The image shows the structure of hemicellulose adapted from (Isikgor and Becer, 2015)

Table 2:1: Types of lignocellulosic biomass and the content of three major component adapted from (Anwar et al., 2014)

| Lignocellulosic material | Lignin (%) | Hemicellulose (%) | Cellulose (%) | Reference[a] |
|---|---|---|---|---|
| Sugar cane bagasse | 20 | 25 | 42 | Kim and Day (2011) |
| Sweet sorghum | 21 | 27 | 45 | Kim and Day (2011) |
| Hardwood | 18–25 | 24–40 | 40–55 | Malherbe and Cloete (2002) |
| Softwood | 25–35 | 25–35 | 45-50 | Malherbe and Cloete (2002) |
| Corn cobs | 15 | 35 | 45 | Prassad et al. (2007) |
| Corn stover | 19 | 26 | 38 | Zhu, Lee, and Elander (2005) |
| Rice straw | 18 | 24 | 32.1 | Prassad et al. (2007) |
| Nut shells | 30–40 | 25–30 | 25–30 | Howard, Abotsi, Van Rensburg, and Howard (2003) |
| Newspaper | 18–30 | 25–40 | 40–55 | Howard et al. (2003) |
| Grasses | 10–30 | 25–50 | 25–40 | Malherbe and Cloete (2002) |
| Wheat straw | 16–21 | 26–32 | 29–35 | McKendry (2002) |
| Banana waste | 14 | 14.8 | 13.2 | John et al. (2006) |
| Bagasse | 23.33 | 16.52 | 54.87 | Guimarães, Frollini, Da Silva, Wypych, and Satyanarayana (2009) |
| Sponge gourd fibres | 15.46 | 17.44 | 66.59 | Guimarães et al. (2009) |

## 2.3 Extraction of Reducing Sugar

### 2.3.1 Pre-treatment (Delignification)

Pre-treatment is a crucial step for the recovery of cellulosic content from lignin-based biomass as compare to the starchy materials. This process is essential in breaking

down the lignin barrier to recover cellulose, which is further subjected to enzymatic hydrolysis to convert into fermentable sugars (Harmsen et al., 2010). During the past few decades, various pre-treatment approaches have been unfolded for carrying out cost-effective fermentable sugar from most of the agrarian cellulose and hemicellulose from lignocellulosic materials. Characteristics of an effective pretreatment is shown by several criteria which are securing portion of hemicellulose, to yield largest contents of fermentable sugar, reduce the loss of carbohydrate, to reduce the formation of inhibitors due to degradation products, curtailing energy input, and the process is economically efficient as well as cost-effective

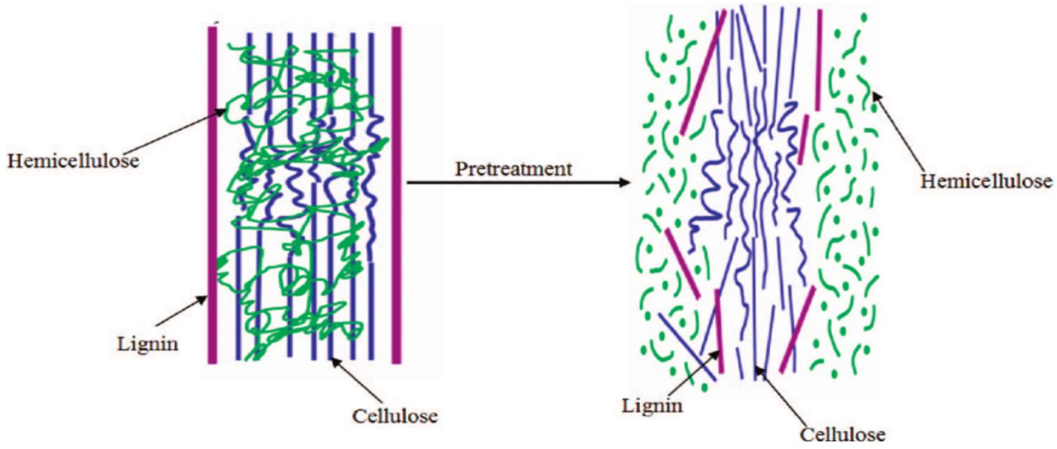


Figure 2:7: The image shows a schematic alteration structure of lignocellulosic biomass adapted from (Kumar et al., 2009)

. Hydrolysis of biomass can be done by different way mainly including physico-chemically, chemically or biologically and choosing the process that interest the feedstocks type should be comprehensively considered as a basis to achieve maximal end product of interest. The most commonly approached way is chemical pre-treatments include acid and alkali based hydrolysis. (Anwar et al., 2014). Table 2.2 shows several types of the pre-treatment that exist and have been researched before.

Table 2:2: Types of delignification processes with its advantages and disadvantages adapted from (Anwar et al., 2014)

| Method of pre-treatment | Sugar yield | Inhibitor formation | Byproduct generation | Reuse of chemicals | Applicability to different feedstock's | Equipment cost | Success at pilot scale | Advantages | Limitations & disadvantages |
|---|---|---|---|---|---|---|---|---|---|
| Mechanical | L | Nil | No | No | Yes | H | Yes | Reduce cellulose crystallinity | High power consumption than inherent biomass energy |
| Mineral acids | H | H | H | Yes | Yes | H | Yes | Hydrolysis of cellulose and hemicellulose. alters lignin structure | Hazardous, toxic and corrosive |
| Alkali | H | L | H | Yes | Yes | Nil | Yes | Removal of lignin and hemicellulose, increases accessible surface area | Long residence time, irrecoverable salts formed |
| Liquid hot | H | H | L | No | – | – | Yes | Removal of hemicellulose making enzymes accessible to cellulose | Long residence time, less lignin removal |
| Organosolv | H | H | H | Yes | Yes | H | Yes | Hydrolyze lignin and hemicellulose | Solvents needs to drained, evaporated, condensed and reused |
| Wet oxidation | H or L | Nil | L | No | – | H | – | Removal of lignin, dissolves hemicellulose and causes cellulose decrystallization | – |
| Ozonolysis | H | L | H | No | – | H | No | Reduces lignin content, no toxic residues | Large amount of ozone required |
| $CO_2$ explosion | H | L | L | No | – | H | – | Hemicellulose removal, cellulose decrystallization, cost-effective | Does not modify lignin |
| Steam explosion | H | H | L | – | Yes | H | Yes | Hemicellulose removal and alteration in lignin structure | Incomplete destruction of ligninecarbohydrate matrix |
| AFXE | H | L | – | Yes | – | H | – | Removal of lignin and hemicellulose | Not efficient for biomass with high lignin content |
| Ionic liquids | H/L | L | – | Yes | Yes | – | – | Dissolution of cellulose, increased amenability to cellulase | Still in initial stages |

L = low; H = high.

### 2.3.1.1 Alkali Pre-treatment

In this pre-treatment process of agricultural lignocellulosic biomasses, base solutions like sodium hydroxide calcium hydroxide(lime) and ammonium hydroxide (Anwar et al., 2014; Harmsen et al., 2010). Lignocellulosic material structures will tend to alter especially lignin part where it will be depleted, swelling up cellulose, degradation of hemicellulose and cellulose partially during treatment process. Corn stove, wheat, switch-grass, coconut, bagasse, and rice straw are the feedstocks that have been proven to benefit from the alkaline delignification technique. Sodium hydroxide were prove as one of solution that show capability to delignify feedstocks such as switch-grass, wheat straw, hardwoods, and soft-woods with less than 26% lignin content (Anwar et al., 2014).

Using alkaline based pre-treatment tends to form salts components that may be assimilated together with biomass and need to be either recycled or eliminated. This Process gives rather mild condition, so reaction times can be extended to bring out their

effectiveness. The benefit of using alkaline based pre-treatment are to prevent over condensation of lignin, especially for lignocellulosic feedstocks that has low lignin content such as softwood and grasses that could draw a high lignin solubility. Degradation of sugars to furfural, HydroxyMethylFurfural (HMF) and organic acids is finite due to the moderate and mild conditions (Harmsen et al., 2010).

A study of the effects of the alkaline pretreatment conditions from (Eliana et al., 2014), the parameters study were temperature, solid to liquid ratio, NaOH concentration and residence time on the degradation of elephant grass was investigated. The results obtained for pretreatment conditions of 120˚C for 1 h with 2 wt.%. NaOH solution and a solid to liquid ratio of 1:20 the highest yield of ethanol was collected, i.e., 26.1 g/L (141.5 mg ethanol/g dry biomass, 95% of theoretical yield).

#### 2.3.1.2 Acid Pre-treatment

Acid hydrolysis process for the degradation of cellulose to glucose has been studied for the past century (Zhang et al., 2011). Diluted acid pre-treatment over concentrated acid has been tested on a few biomass feed-stocks like grassier materials, agrarian wastes and hardwood. This is because diluted acid benefits the sustainability of environment a little bit where to reduce the issues like acid resumption, toxicity, acid and special maintenance against corrosion materials. The substrates from diluted acid delignification usually gives better outcomes by solubilizing the hemicellulose and free up the cellulose-hemicellulose-lignin bond to further process on recovery of glucose. There were also factors that would greatly affects the lignin and hemicellulose breakdown and they are temperature of hydrolysis and hydrolysis time which giving impact on alteration the structure of biomass. Formation of secondary products such as conversion of products further to furfural and hydroxyl-methyl furfural compounds is the primary disadvantage of this process. It will

interfere the bioethanol fermentation process which can lower the yield of sugars (Anwar et al., 2014).

From other source like (El-Zawawy et al., 2011), pre-treated rice straw, banana plant waste and corn cob were used as cellulosic sources for the ethanol production. Sulfuric acid was used with various concentration such as 4%, 5% and 10%, were initially tested to find out the best concentration and it was carried out at several times differ from 30 mins to 180 mins until they come to a conclusion to use 5% sulfuric acid for 2 h hydrolysis. At the end of the hydrolysis time, the glucose concentration was measured (El-Zawawy et al., 2011). In (Bujang et al., 2013), the condition used are 4g of dry was mixed with 200 mL of 1% sulfuric acid at 130°C at various hydrolysis times which are 30mins, 60mins and 90mins.

### 2.3.2 Direct Acid hydrolysis

The use of concentrated acid such as Hydrochloric Acid or Sulfuric Acid was considered as a conventional procedure. The entire process of hydrolysis can be done at slightly lower temperature as to dilute-acid pre-treatment. However, higher concentration around 30-70% (volume/volume) % might possibly require to balanced up for the lower temperature and pressure condition as a drawback of the hydrolysis. But, it does cause prominent level of corrosive reaction. In comparison to the other pre-treatment procedures particularly dilute-acid hydrolysis the environmental hazards and high operating cost involved in concentrated-acid hydrolysis reduce the interest on industrial scale (Anwar et al., 2014; Kumar et al., 2009). But on the other hand, if the concentrated acid is used as direct acid hydrolysis, there will be less operating cost in pre-treatment since the process will not be required anymore.

While that, (Harmsen et al., 2010) say that concentrated strong acids have been widely used for utilizing lignocellulosic materials because they are potent solution for cellulose degradation. So, there will be no enzymes hydrolysis required for fermentable

sugar production and cost will be reduced. The benefits of using concentrated acid hydrolysis is that they are adaptable in terms of feedstock selection, recover high content of reducing sugar as well as to get mild temperature conditions. However, the flaw from using concentrated acids are the reaction process are very corrosive and in order to reduce the cost, the acid will need to be recycled (Harmsen et al., 2010).

## 2.4 DNS Calorimetry Analysis

3,5-Dinitrosalicylic acid or known as DNS reagent is a common practice to estimate the content of reducing sugar sugars and it is an assay that was approved by the International Union of Pure and Applied Chemistry (IUPAC) (Saqib and Whitney, 2011). It is called Miller Calorimetry method where the reagent shows a particular feature towards monosaccharides and disaccharides. Reducing sugars are known from the existence of free carbonyl group (C=O), in which DNS calorimetry method will figure that out. Reducing sugar represent as reducing agents which held an aldehyde functional group or ketone functional group and in form of an open chain structure (Saqib and Whitney, 2011; Wang, 2017).

The process involves the oxidation of the aldehyde functional group held in sugar sample, such as glucose and fructose from ketone functional group. At the same time, 3,5-Dinitrosalicylic acid is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions as shown below in (2.4a) and (2.4b):

**Oxidation**

aldehyde group ----------> carboxyl group (2.4a)

**Reduction**

3,5-dinitrosalicylic acid ----------> 3-amino,5-nitrosalicylic acid (2.4b)

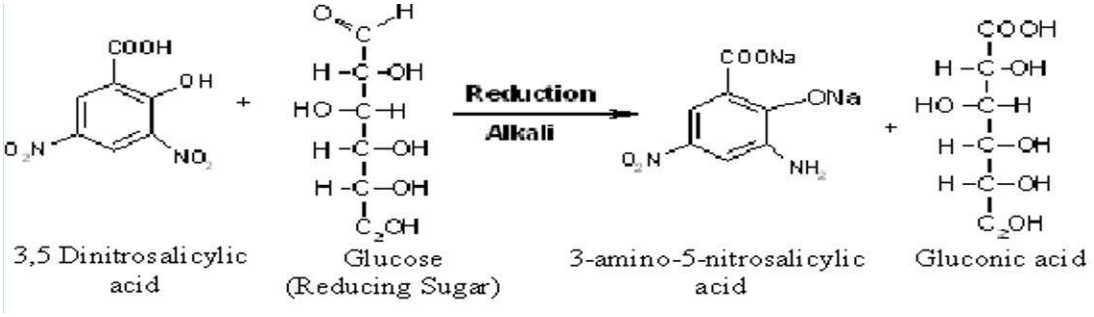


Figure 2:8: shows diagram of (2.4a) + (2.4b) reaction adapted from (Wang, 2018).

## 2.5 Past Research Journal's Outcome

There is still lack the study of utilizing coconut dregs s a useful product for certain industries. However, the journal of (Bujang et al., 2013) has some relation in this project work. Figure 2.9 shows the reducing sugar yield by (Bujang et al., 2013) where, the sample of 4g coconut dregs were subjected to diluted acid hydrolysis namely 1% concentration of $H_2SO_4$ solution at 130℃ and for various hydrolysis time of 30mins, 60mins and 90 mins. The medium of heating used are autoclave and the parameter were only hydrolysis time. The analysis of reducing sugar was conducted by using Biochemistry analyzer. Highest yield of 0.38 g/l glucose concentration was obtained at the time of 60 min.

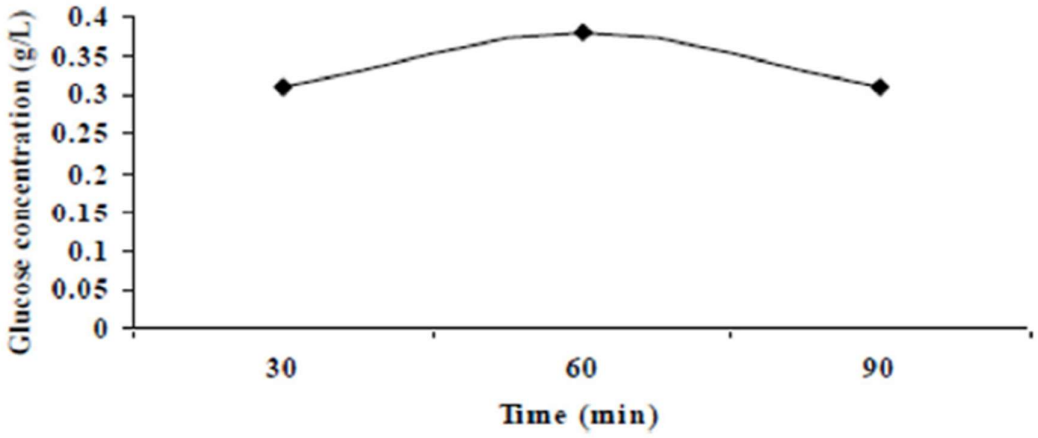


Figure 2:9: Result reducing sugar recovery with hydrolysis time as parameter adapted from (Bujang et al., 2013)

In comparison to this project, the process of extracting the raw materials for producing bio-ethanol and bio-cokes/bio-reductant are going through pre-treatment and then followed by indirect/direct hydrolysis study. The initial condition for testing is 0.5% concentration of acid and base solution. Several parameter is set up with one factor at a time method (OFAT) consists of amount of sample, hydrolysis time, hydrolysis temperature, solid to liquid ratio and concentration of acid. The medium of heating used in this project is oil bath. The analysis of reducing sugar conducted by using Dinitrosalicylic acid (DNS) calorimetry method measured with UV-Visible Spectrometry.

Figure 2.10 shows the content of major composition done by (Bainun et al., 2017) where in this project, there is no composition study done to the coconut dregs. The content of lignin varies greatly with different type of lignocellulosic biomass as in Table 2.1. So, for the delignification process, this proximate composition percentage will be adapted.

Proximate analysis of coconut dregs

| Composition | Percentage, % |
|---|---|
| Crude protein | 6.54 |
| Crude fibre | 27.33 |
| Crude fat | 9.35 |
| Cellulose | 36.08 |
| Hemicellulose | 12.58 |
| Lignin | 9.81 |

Figure 2.10: Shows the proximate analysis of coconut dregs adapted from (Bainun et al., 2017)

CHAPTER 3

# METHODOLOGY

## 3.1 Overview

This chapter describes the used of raw materials, preparation of samples, types of testing and procedures of the experiment. In general, this work consists of three major sections which are characterization of coconut dregs before and after hydrolysis to observe their surface morphology, delignification process, indirect and direct hydrolysis of coconut dregs, and analysis of hydrolyzed solution of coconut dregs for determination of glucose contents.

Hydrolysis process are divided into 3 types hydrolysis that is being tested on several parameters. They are pre-treatment hydrolysis and, indirect and direct acid hydrolysis. Generally, the experimental work method is one factor at a time (OFAT). The parameters that play the most important role are hydrolysis time, Temperature, Solid and Liquid ratio, and Concentration of acid. As for analysis, Calorimetry method Dinitro salicylic Acid (DNS) is being adapted to determine the quantitative sugar content.

Overall of the project experiment mainly conducted in Chemical Laboratory and Analytical Laboratory with technicians' observation and guidance, En. Azrul and Pn. Haslina. Figure 3.1 shows the general flow of the project.

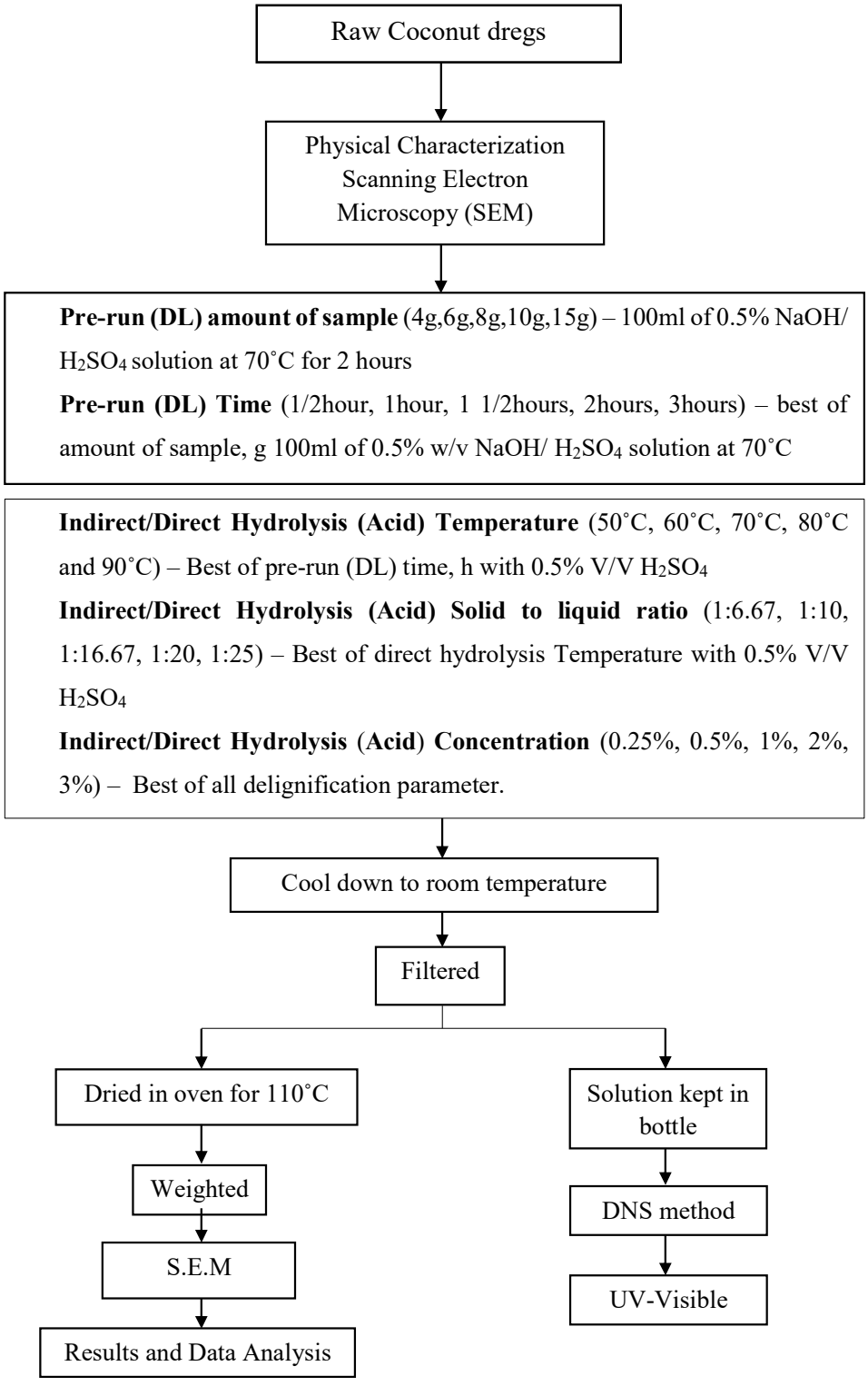


Figure 3:1: Shows the whole flowchart of the hydrolysis of coconut dregs.