PRODUCTION OF XYLOSE FROM OIL PALM FROND FIBRE BY ACID HYDROLYSIS

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Thesis is submitted in partial fulfilment of the requirements
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This thesis is dedicated to my father, Mr. Maniarasan Gopal and my mother Mrs. Puvanesvari Velappuly

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ABSTRACT

Oil palm frond fibre (OPFF) is one of the oil palm biomass which is available in a large amount worldwide in oil palm plantation. Oil palm frond fibre (OPFF) is cheap, widely available, and has high potential to be a renewable source for the production of xylose. This paper presents the production of xylose from oil palm frond fibre by acid hydrolysis. The presence of xylose was tested using DNS analysis. In this research, diluted acid hydrolysis was applied for the treatment of oil palm frond fibre (OPFF) and several parameters including temperature (70, 85, and 100 °C), acid concentration (1, 3, and 5 M), and solid to liquid ratio (0.5 g:10 mL, 0.5 g:12.5 mL, and 0.5 g:15 mL) were taken into account in order to get the optimum conditions for xylose recovery from oil palm frond fibre (OPFF). The optimum xylose obtained was 1.0183 mg/mL under optimal conditions with acid concentration of 5M, temperature of 70 °C, and a solid to liquid ratio of 0.5 g:15 mL. Findings from this work is vital to produce xylose as the raw material for the production of xylonic acid and to make sure that we could find a solution for the abundance of oil palm frond fibre which might lead to environmental issues such as greenhouse gas emission and global warming.

ABSTRAK

Serat pelepah kelapa sawit (OPFF) adalah salah satu daripada biomas kelapa sawit yang boleh didapati dalam jumlah yang besar di seluruh dunia. Serat pelepah kelapa sawit (OPFF) adalah murah, mudah diperolehi, dan mempunyai potensi yang lebih tinggi untuk menjadi sebagai sumber yang boleh diperbaharui untuk pengeluaran xylose. Kertas kerja ini membentangkan pengeluaran xylose daripada gentian pelepah kelapa sawit oleh kaedah hidrolisis asid. Kehadiran xylose telah diuji menggunakan analisa DNS. Dalam kajian ini, hidrolisis asid akan digunakan untuk rawatan gentian pelepah kelapa sawit (OPFF) dan beberapa parameter termasuk suhu (70, 85, dan 100 °C), kepekatan asid (1, 3, dan 5 M), dan nisbah pepejal kepada cecair (0.5 g:10 mL, 0.5 g:12.5 mL, dan 0.5 g:15 mL) diambil kira untuk mendapatkan keadaan optimum bagi perolehan xylose daripada gentian pelepah kelapa sawit (OPFF). Sebanyak 1.0183 mg/mL diperolehi melalui proses hidrolisis dengan kepekatan asid 5M, suhu 70 °C, dan nisbah pepejal kepada cecair 0.5 g:15 mL. Dapatan kajian ini adalah penting untuk menghasilkan xylose sebagai bahan mentah untuk penghasilan asid xylonic dan memastikan bahawa kita dapat mencari penyelesaian bagi lambakan serat pelepah kelapa sawit yang mungkin membawa kepada isu-isu alam sekitar seperti pelepasan gas rumah hijau dan pemanasan global.

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M	Molarity
°C	Degree Celsius

LIST OF ABBREVIATIONS

MPOB	Malaysian Palm Oil Board
OPFF	Oil Palm Frond Fibre
EFB	Empty Fruit Bunches
OPT	Oil Palm Trunk
POME	Palm Oil Mill Effluent
H_2SO_4	Sulphuric Acid
HCl	Hydrochloric Acid
NaOH	Sodium Hydroxide
TRS	Total Reducing Sugar
SEM	Scanning Electron Microscope Analysis
TGA	Thermogravimetric Analysis
FTIR	Fourier transform infra-red spectrometry
DNS	3,5-Dinitrosalicylic Acid
OD	Optical Density

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Oil palm frond fibre (OPFF) have been reported as suitable biomass for biochemical and biomaterials production since these materials are rich in carbohydrate and lignin, available throughout the year, renewable and sustainable sources.(Zahari *et al*., 2014). Biomass as a renewable energy source can reduce the dependency on fossil fuels and provide significant advantages in terms of carbon dioxide emissions reduction and therefore less greenhouse effect will be seen. Oil palm frond fibres (OPFF) are the most abundant type of wastes produced worldwide with annual generation capacity of about 58 million tonnes in 2011(Nafsiah *et al*., 2013).

OPFF is chosen as the raw material because it is one of the renewable sources which is rich in lignocellulosic material that is possible to be converted into value-added products and chemical. Besides that, the uses of lignocellulosic material may help to cut the overall cost of production because this material is cheap and easily available. Moreover, the environmental problem may reduce if lignocellulosic material is used as raw material.

On the other hand, lignocellulosic material has three main components which are hemicelluloses, cellulose and lignin. Hemicelluloses and cellulose are referred as carbohydrate polymer while lignin as aromatic polymer. The cellulose component is known as glucose polysaccharides while hemicelluloses are divided into two type of polysaccharide with different sugars which are hexoses (glucose, mannose and galactose) and pentoses (xylose and arabinose) (Wong *et al*., 2011) .

The lignocellulosic material undergoes acid or enzymatic hydrolysis in order to release xylose from hemicelluloses. For this research, acid hydrolysis was chosen rather than enzymatic hydrolysis because acid hydrolysis is an easy method compared to enzymatic hydrolysis. Many studies show that, acid treatment can be more dependable for the hemicellulosic fraction to be hydrolysed. The efficient utilization of these wastes for value added bio-products would not only save the environment but also increase the economic income for nation as the aim of this research is to produce xylose from oil palm frond fibre.

The amount of xylose recovered from the OPFF depends on the liquid to solid ratio of acid to biomass, temperature, and concentration of acid. It is important to determine the suitable condition of acid hydrolysis in order to get a high value of xylose production.

1.2 Motivation

The palm oil industry is the backbone of the Malaysia's economic growth. Indisputably, Malaysia is an essential source of this commodity. In 2014, 25.07 million tonnes of oil palm products from Malaysia were exported abroad (Fahmi *et al.*, 2014). During oil palm fruit harvesting, the pruning of OPFF produces approximately 44 million tonnes dry weight of OPFF annually.

Malaysia has been the one of the world leading producer for oil palm industry. According to latest statistic on April 2014 shown by Malaysian Palm Oil Board (MPOB), oil palm trees cover a total of 5.230 million hectare representing 79% of the total area (6.6 million hectare, 2009) designated for agriculture and 15% of overall Malaysian territory (Kong *et al.*, 2014).

According to the total life time of oil palm tree plantation, only 10% by weight of oil palm tree is converted as the final product which are palm oil and kernel oil while the remaining 90% will become biomass waste in the form of empty fruit bunches (EFB), kernel shells, palm oil mill effluent (POME), trunks and oil palm frond fibres (OPFF) (Kong *et al*., 2014).

The waste generated from oil palm plantation in recent years have created a major disposal problem due to the fact that open burning of the wastes are discouraged by the Department of Environment in Malaysia. Hence, the conversion of these wastes into value added products such as xylose using acid hydrolysis method will overcome the aforementioned problems. Production of xylose from OPFF is mainly used for the production of xylonic acid in industries.

1.3 Problem Statement

The amount of organic waste obtained from the agriculture industry is abundant in Malaysia but the utilization is still limited. Despite the tremendous availability and potential commercial exploitation, oil palm frond fibres (Wanrosli *et al*., 2004) have rarely received any particular attention from investors. This may be partially due to the fact that these biomasses are not directly or closely involved in the palm oil milling process, as compared with empty fruit bunches.

Oil palm frond fibres can be found usually after harvesting and replanting activities which can be considered as an abundant waste material produced by the palm oil industry in Malaysia (Zahari *et al*., 2014). This waste has become worrisome in oil palm industry because it affects the environment besides it may lead to greenhouses gas emission and global warming if do not treated properly.

The traditional method such as open dumping, burning and land filling of these materials for disposing purposes are not only increasingly expensive but also impractical due to limited open space. It is beneficial to use oil palm frond fibres as a raw material in the production of xylose because it may overcome this problem. In increased of health problem and rising health concern, modern world has promoted various medicinal and natural products.

Xylose is very important since it is well known as natural sugar substitute and commercially used for various applications such as sweetener in food industry, healthcare sectors, pharmaceuticals, animal nutrition and also alternative sweetener for diabetic patients. It has higher demand because public have realized how important it is and also have much higher awareness about health issues. Besides that, xylose production from chemical process is expensive and also requires high production cost. However,acid hydrolysis is becoming more popular because it costs lower and have greater effectiveness than enzymatic hydrolysis (Taherzadeh *et al*., 2007 and Karimi *et al*., 2007)

1.4 Objective

This research aims to recover xylose from oil palm frond fibre (OPFF) using acid hydrolysis by varying

- The temperature on the acid hydrolysis of biomass to xylose
- The concentration of acid
- The liquid to solid ratio of acid to biomass

1.5 Scopes of study

The scope of the study will be focused

- To investigate the effect of temperature ranging from (70 °C, 85 °C, and 100 °C) on the acid hydrolysis of biomass to xylose.
- To determine the effect of concentration (1M, 3M, and 5M), of the acids involved in acid hydrolysis process.
- To identify the effect of solid to liquid ratio (0.5 g:10 mL, 0.5 g:12.5 mL, and 0.5 g:15 mL) of acid and biomass.

CHAPTER 2

LITERATURE REVIEW

2.0 Overview

Raw material used in this research is oil palm frond fibre (OPFF) which is one of the most abundant biomass waste found in oil palm plantation in Malaysia. The use of OPFF is possible as it is one of the lignocellulosic material. Lignocellulosic material has three components which are cellulose, hemicelluloses and also lignin. Lignin and hemicellulose creates a shield for cellulose against of the physical, microbial or chemical degradation and restricts the significant swelling of the cell wall, thereby restricting accessibility of cellulose to the acid (Eckard *et al.*,2012).

Pretreatment is necessary as it removes hemicelluloses and lignin that limit the penetration of acid to the cellulose while hydrolysis of acid is being carried to extract the xylose. Dilute acid hydrolysis is one of the reliable methods for this purpose and from this acid hydrolysis treatment, xylose sugar can be produced to be used as a substrate for the production of natural sweetener of xylose.

2.1 Oil palm tree

The oil palm tree is a tropical palm tree under the family of Palmae originally came from Guinea, West Africa (Yusof *et al*., 2000). It grows well in all tropical areas of the world and has become one of the main industrial crops. Oil palm tree was first time introduced in Malaysia during years of 1870 as an ornamental plant. The most suitable area for the growth of oil palm tree is soil with low pH and the life span of a single tree is about 25 years.

Oil palm tree consists of trunks, bunches and fronds. A bunch of fruit weighs about 10 to 25 kg and sometimes might up to 50kg and most suitable time for harvesting ripe fruits are from 5 to 6 months after the flowering process. Oil palm tree has become one of the most important plants in Malaysia and main important contributor to Malaysian economy and also known as the world’s second largest palm oil producer after Indonesia (Abdullah *et al*., 2009). Malaysia planting of oil palm commercially began in year 1917 and since then, the growth of the industry in terms of planted area has been very rapid.

The total area planted with oil palm was 3,499,012 hectares, 59.9% or 2,096,856 hectares being in Peninsular Malaysia, 29.4% or 1,027,329 hectares in Sabah and 10.7% or 374,828 hectares in Sarawak in 2001 (Teoh *et al*., 2002). Besides that, according to MPOB (2012), Malaysia had 5 million hectares of oil palm areas in year of 2011. Figure 1.0 shows the oil palm production in Malaysia from year 2004 to 2009.

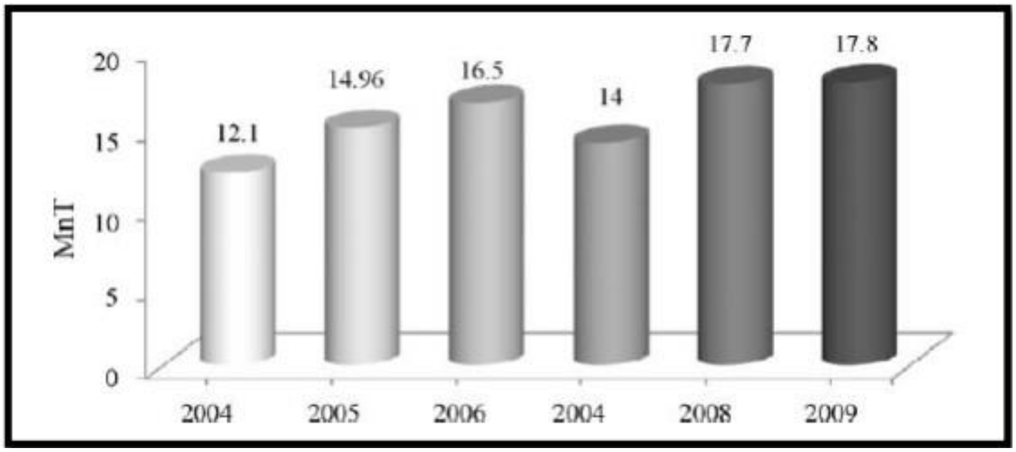


Figure 1.0: Palm oil production in Malaysia from 2004 – 2009

Million tonnes per year of biomass are being produced from oil palm industries, which when treated properly, it will not only be able to solve the disposal problem but also can create value added products for a better future (Shuit *et al*., 2009). There are many types of biomass produced from oil palm industry which are empty fruit bunches (EFB), palm oil mill effluent, fiber, shell, wet shell, palm kernel, oil palm frond fibres (OPF) and oil palm trunks (OPT). Table 2.1, shows the percentage of waste of oil palm tree annually.

Table 2.1: Waste of oil palm (Kong *et al.*, 2014).

Products /Waste	**Percentage by weight % (dry basis)**
Palm oil	21
Palm kernel	7
Fibre	15
Shell	6
Empty fruit bunches	23
POME	28
Total	**100**

2.2 Properties of Oil Palm Frond Fibres

The raw material which will be used in the production of xylose via acid hydrolysis is oil palm frond fibre also known as OPFF. The average density of oil palm frond is about 700 kg/m3. The weight of each OPFF is between 15 kg and 20 kg depending on the age and condition of the palm tree. The OPFF comprises of two main components which are petiole and leaflets. The dry matter weight ratio of petiole to leaflets (including the rachis) is 1.5 (Sulaiman *et al.*, 2014). Typically, the main biomass composition is made up of carbon and it comprises 30% to 60% of dry matter.

As mentioned earlier, oil palm frond fibre biomass is lignocellulosic organic material which is composed mainly by three components which are cellulose, hemicellulose, and lignin. These organic components are incorporated to form a strong cellulose-hemicellulose-lignin complex structure within the plant material. In order to get maximum access to cellulose, this cellulose-hemicellulose lignin structure must be destroyed by physical, chemical, or biological treatments. Such pretreatment is a crucial step before acid hydrolysis is carried out.

Table 2.2: Components of lignocellulosic biomass in the waste of oil palm (Harmsen *et al.*, 2010).

Components	**Oil palm trunk**	**Oil palm fronds**	**Empty fruit bunch**
Lignin	18.1	18.3	21.2
Hemicellulose	25.3	33.9	24.0
a-cellulose	45.9	46.6	41.0
Holocellulose	76.3	80.5	65.5
Ash	1.1	2.5	3.5
Alcohol-benzene solubility	1.8	5.0	4.1

2.2.1 Lignocellulose

Lignocellulose is a class of biomass that consists of three major compounds cellulose, hemicellulose and lignin. It also includes water and a small amount of proteins and other compounds, which do not participate significantly in forming the structure of the material (Raven *et al.*, 1992). Inside the lignocellulose complex, cellulose retains the crystalline fibrous structure and it appears to be the core of the complex. Hemicellulose is positioned both between the micro- and the macrofibrils of cellulose. Lignin provides a structural role of the matrix in which cellulose and hemicellulose is embedded (Faulon *et al.*, 1994).

2.2.2 Cellulose

Cellulose is an organic compound with the formula of $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to many thousands of β linked D-glucose units (Raven *et al.*, 1992)**.** In cellulose, the bond will be linked by hydrogen from hydroxyl groups on the glucose with oxygen atoms on the same or on a neighbour chains firmly together side by-side. This bond makes cellulose results in a high tensile strength, crystalline, strong and difficult to depolymerize. Cellulose chain length depends on total glucose units that were linked to form polymer such as wood pulp with 300 to 1700 units, cotton and other plant fibers with a chain length from 800 to 10,000 units.

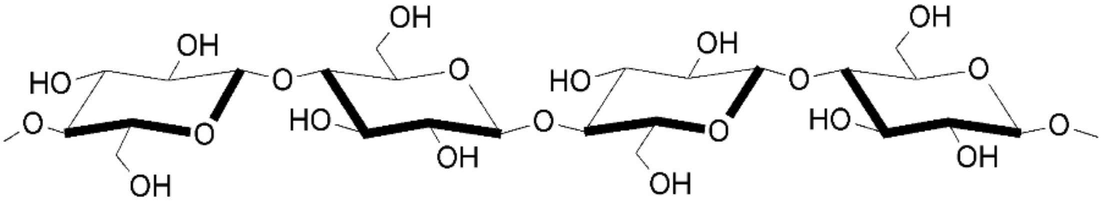


Figure 2.0: The chemical structure of cellulose.

2.2.3 Hemicellulose

Hemicellulose represents a family of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and other elements which can be found in the plant cell wall besides have different composition and structures depending on their source and the extraction method. Hemicelluloses are heteropolymers with matrix polysaccharides in almost all plant cell walls and have a random structure of branched sugars polymer such as xylose and arabinose. In hemicellulose, xylose is a major D-pentose sugar monomer followed by L-sugars of arabinose. In addition, hemicellulose also contains small amount of mannuronic acid and galacturonic acid (Girio *et al*.,2010).

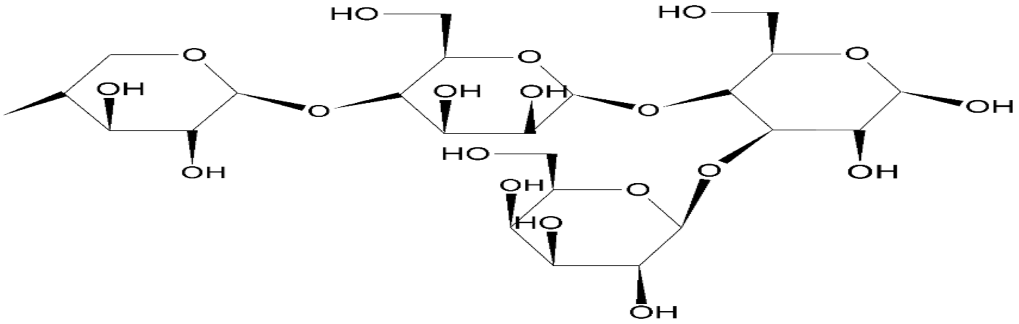


Figure 2.1: The chemical structure of hemicellulose.

2.2.4 Lignin

Lignin is a class of complex organic polymers that form important structural materials in the support tissues of oil palm tree and also other vascular plants. Lignins are particularly important in the formation of cell walls, especially in wood and bark, because they lend rigidity and do not make them to rot easily. Chemically, lignins are cross-linked phenolic polymers. Lignin is an amorphous highly complex aromatic structure polymer connected mainly by three-dimensional cross-linked C-C and C-O-C of phydroxyphenylpropanaid units. It is hydrophobic and can be classified by its variable structural element. Lignins are soluble in base solution which makes it easy to be removed from lignocellulose plant biomass.

Table 2.3: Sugar content of different plant holocellulose (Park *et al*., 2012 and Kim *et al*., 2012).

Lignocelluloses material	**Eucalyptus (%)**	**Larix Leptolepis (%)**	**Pinus Rigida (%)**	**Rice Straw** (%)	**Barley Straw (%)**
Cellulose	41.8	43.4	43.1	39.1	35.9
Hemicellulose	18.7	24.4	23.7	23.6	29.1
Lignin	30.1	28.9	29.0	12.1	15.4
Others	9.4	3.3	4.2	25.2	19.6

Acids cannot efficiently convert the lignocellulosic biomass into xylose due to the complex structure of lignocellulose in plant. A variety of pre-treatment methods need to be applied towards the lignocellulosic feedstocks in order to enhance the sugar yield. The production of xylose from cellulosic biomass generally follows well-established practices such as grinding and milling of crop residues, chemical pre-treatment and chemical hydrolysis process (Chandel *et al*., 2007).

2.3 Method of pretreatment

Pretreatment is a crucial process step for the conversion of lignocellulosic biomass into some value added products such as xylose. The pretreatment process is carried out to remove lignin and hemicellulose that reduces the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment that is being carried out should be able to subsequently form sugars by hydrolysis, avoid the degradation or loss of carbohydrate, avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis (Kumar *et al.*, 2009). Pre-Treatment methods are divided into three parts which are Chemical, Physical, Biological.

2.4 Chemical Method

Table 2.4: Chemical methods for biomass lignocellulosic pretreatment.

Chemical Pretreatment Method	**Conditions**	**Advantages**	**Disadvantages**	**Resources**
Concentrated acid eg : H_2SO_4, HCl	Concentrated acid Low temperature	High yield	Corrosion problem high cost formation of toxic substances	Saha et al. (2005)
Dilute acid Eg: H_2SO_4	W = 0.5-2% T > 150 °C	Well known and ready used	Corrosion problem Low yield	Saha et al. (2005)
Ammonia Eg : Ammonia fibre explosion-method (AFEX), Ammonia recycle percolation (ARP)	W = 15% T = ~ 170 °C	Media recoverable	Environmental issues	Galbe*et al.* (2007) and Zacchi *et al.* (2007)

Chemical Pretreatment Method	**Conditions**	**Advantages**	**Disadvantages**	**Resources**
Alkaline hydrolysis eg :NaOH	W = 0.5-2% T > 150 °C	None	Long residence times required, irrecoverable salts formed	Kumar *et al*. (2009)
Organosolv	T =150 °C - 200 °C	Cost of solvent Media recoverable	solvents need to be drained from the reactor, high cost	Zhao *et al*. (2009)
Room Temperature Ionic Liquids salts	T=24°C (room temperature)	Low temperatures No toxic or explosive gases are formed	Residues removal would be required	Li *et al*. (2009)

2.4.1 Concentrated Acid Hydrolysis

Concentrated acid hydrolysis of lignocelluloses usually yields a near-theoretical sugar value but with fewer degradation products. The most widely used and tested mineral acid for the hydrolysis process is hydrochloric acid, sulphuric acid and also phosphoric acid. The concentrated acid hydrolysis uses relatively mild temperatures, but is conducted at a very high concentration of sulphuric acid and at a minimum pressure (Girio *et al*., 2010).

This process provides complete and rapid conversion of cellulose to glucose and hemicellulose to xylose with low degree of degradation (Lenihan *et al*., 2010). High sugar recovery efficiency is the primary advantage of the concentrated acid hydrolysis process. Approximately 90% of cellulose and hemicelluloses are degraded to their sub units after the treatment process. The low temperature and pressure of the concentrated acid hydrolysis, leads to minimised sugar degradation (Chandel *et al*., 2007).

2.4.2 Dilute Acid Hydrolysis

Dilute acid hydrolysis of biomass has successfully degraded lignocelluloses materials into sugars. Dilute acid hydrolysis is a simple process and no acid recovery is needed. The process needs an optimum condition like high pressure and temperature to break down the cellulose to glucose and to prevent lower yields of sugar from being produced. Compared with concentrated acid, dilute acid is relatively low acid consumption and causes less corrosion of the equipment (Girio *et al*., 2010).

Dilute acid hydrolysis is conducted at two stages due to the different structure of cellulose and hemicellulose. In the first stage the process needs a milder condition to hydrolyze hemicelluloses followed by the second stage where the process needs a much harsher condition to hydrolyze cellulose. The advantage of dilute acid is to avoid formation of inhibitors such as furan compound, weak carboxylic acids and phenolic compounds which can reduce yield of ethanol during fermentation of sugars.

2.4.3 Ammonia Treatment

The aqueous ammonia treatment reaction mechanism is similar to lime treatment where the biomass swelling and cleave the ether and ester bonds in lignin. Aqueous ammonia can solubilize lignin to 65-85% without degradation of cellulose fraction and loss of glucan. Aqueous ammonia is known to remove lignin and to enhance saccharification and acid hydrolysis of cellulose to sugar for production of ethanol (Jung *et al*., 2011).

2.4.4 Organosolvation

The organosolvation method is a promising pretreatment strategy, and it has attracted much liquid to solid ratio ranging from 4:1 to 10:1 (w/w). The pH of the liquid might range from 2.0 to 3.8. The largest component, which is cellulose, will be partially hydrolyzed into smaller fragments that still remain insoluble in the liquid. The second largest component, hemicellulose, will be hydrolyzed mostly into soluble components, such as oligosaccharides, monosaccharides, and acetic acid. Acetic acid lowers the pH of liquid, stimulating acid-catalyzed hydrolysis of other components. Some of the pentose sugars are subsequently dehydrated under the operating conditions to form furfural. The third major polymer component lignin, is hydrolyzed under the conditions employed in the process primarily into lower molecular weight fragments that dissolve in the aqueous ethanol (Zhao *et al*., 2009).

2.4.5 Alkaline treatment

The alkaline treatment can be done by using alkaline earth metals based agents such as ammonia. Most lime treatment methods will use sodium, calcium and potassium. Alkaline treatment readily removes lignin and increases efficiency of enzyme saccharafication compared to acid treatment (Sun *et al*.,2002 and Cheng *et al*.,2002). However, without proper delignification, there will be loss of some of the sugars (Yadav *et al*., 2011). Calcium hydroxide and sodium hydroxide are the most common lime used for pre-treatment because they are relatively low-cost and safer reagent compared to other alkalis (Kaar *et al*.,2010 and Holtzapple *et al*., 2010).

2.5 Physical Treatment

Table 2.5: Physical method for biomass lignocellulosic pretreatment.

Physical Pretreatment Method	**Conditions**	**Advantages**	**Disadvantages**	**Resources**
Steam-Explosion	P =2.5-7MPa T=180 °C-280 °C	Well known High yield	Undesired side product High energy demand	Kumar *et al*. (2009)
LHW- Liquid Hot Water	T = 170 °C – 230 °C	Low side product	None	Pérez *et al*. (2008)
CO2 Explosion	P > 7.3 MPa T > 31.1 °C Super critical CO2	Effectively remove lignin	High cost	Schacht *et al*. (2008)
AFEX Ammonia Fiber Explosion	Liquid Ammonia T = 90 °C-100 °C	Low inhibitor formation	Environmental impact	Kumar *et al*. (2009)
Milling /Grinding	Size : < 0.85mm Time : 3-120 min	Reduction of particle sizes Reduction of crystalline No chemicals are required	High energy	Sun *et al*. (2002) and Cheng, *et al*. (2002)

2.5.1 Steam explosion

Steam explosion is the most commonly used method for pre-treatment to breakdown the structural component by heat in the form of steam and force shear. Initially, the chipped material wetting will take place for several seconds to a few minutes at a temperature range of 160 °C–260 °C with pressure 0.69 - 4.83 MPa. After several seconds to 18 minutes, pressure will be released to the atmospheric pressure and biomass structure will explode.

The desegregation of inter and intra molecular linkage causes hemicellulose degradation and lignin transformation to cellulose hydrolysis. The efficiency of this process depends on several factors such as the rate of reaction, temperature, size of biomass and moisture content. The process can have a higher conversion either at a high temperature of 270 °C for 1 min or lower temperature of 190 °C for 10 min (Girio*et et al*., 2010, Moelhman *et al*., 2010).

2.5.2 Liquid hot water

Hydrothermal is the process of using liquid hot water (auto hydrolysis) and steam explosion treatment (Cristobal *et al*., 2007). The process involves the compression of hot air into material with a temperature of 150 °C – 230 °C (Garrote *et al*., 1999). The reaction will take place when hydronium ions generate in-situ by water auto ionization.

2.5.3 Carbon Dioxide Explosion

In attempts to develop improved lignocellulose pretreatment techniques, the idea of using supercritical CO_2 explosion, which would have a lower temperature than steam explosion and possibly would reduce the expense compared to ammonia explosion. Supercritical fluid refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point (George *et al*., 1995). It is hypothesized that, CO_2 will form carbonic acid when dissolved in water and thus the acid will increase the hydrolysis rate.

Carbon dioxide molecules are comparable in size to water and ammonia and thus it should be able to penetrate small pores which are accessible to water and ammonia molecules. Carbon dioxide was suggested to be helpful in hydrolyzing hemicellulose as well as cellulose. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid. After an explosive release of the carbon dioxide pressure, the disruption of the cellulosic structure increases the accessible surface area of the substrate to hydrolysis. (Dale *et al*., 2009).

2.5.4 AFEX

AFEX is similar to steam explosion where a process will need pressure to break lignocellulose structure. Typically, AFEX process will be conducted at 90 °C in residence time for 30 minutes. It will be used for the pre-treatment with low lignin content in lignocelluloses materials such as wheat straw, wheat chaff, rice straw (Vlasenko *et al*., 1997), kenaf newspaper (Holtzapple *et al*., 1992), switchgrass (Reshamwala *et al*.,1995), aspen chips and bagasse (Holtzapple *et al*.,1991). In addition, small particle size of biomass is not required in AFEX pretreatment compared to other pre-treatment processes (Ming Lau *et al*., 2010).

2.5.5 Grinding

The biomass is required to undergo a necessary size reduction in order to make it adequate for further downstream processing. The particle size of the biomass will influence the penetration of acid into OPFF biomass particle (Najaf *et al*., 2007). Practically, physical pre-treatment will be conducted by mechanical process such as chipping, grinding and explosion. A combination of chipping, grinding and milling in biomass will reduce cellulose crystalline for further process. Generally, the size of the biomass materials will be from 10–30 mm after chipping and 0.2–2 mm after milling or grinding.

2.6 Biological method

Table 2.6: Biological method for biomass lignocellulosic pretreatment.

Biological Pretreatment Method	**Example of organism**	**Advantages**	**Disadvantages**	**Resources**
Fungi	White-rot Fungi Brown-rot Fungi Soft-rot Fungi	Low energy requirement No chemical required	Slow conversion Mild environment conditions	Sánchez *et al*. (2009)
Bacteria	Sphingomonas Paucimobilis Bacillus circulans	Low energy requirement No chemical required	Slow conversion Mild environment conditions	Verardi *et al*. (2014)

Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase.These enzymes are regulated by carbon and nitrogen sources.White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials. The pretreatment of wheat straw by 19 white-rot fungi and found that 35% of the straw was converted to reducing sugars by *Pleurotus ostreatus* in 5 weeks.

Despite the higher energy requirement, grinding pretreatment of lignocellulosic residues has a great impact on the reduction of particle sizes by changing the cellulose crystalline structures and chemical bonding distortions due to imposed stress, as well as decreases in crystallinity and degrees of polymerization of cellulose. Furthermore, this process is simple yet no additional chemicals are required and no other functional groups changes will be involved over grinding time. Efficient pretreatment by this process will be demonstrated by the high recovery of xylose, and total conversion yields from acid hydrolysis of treated oil palm frond fibres (Zakaria *et al*., 2014).

Table 2.7 Archea optimum condition

Archea					
Enzymes	**Organism**	**pH optimum**	**Optimum temperature**	**Stability (half life)**	**Reference**
B-glucosidase	Pyrococcus Furiousus	5	102	13 h at 110 °C	Adams *et al*. (1994)
	Pyrococcus horikoshi	6	100	15 h at 90 °C	Rahman *et al*. (1998)
Endoglucanase	Pyrococcus Furiousus	6	100	40 h at 95 °C	Bergquist *et al*. (2004)
	Pyrococcus horikoshi	6-6.5	100	19 h at 100 °C	Bergquist *et al*. (2004)

Table 2.8 : Bacteria optimum condition

Bacteria					
Enzymes	**Organism**	**pH optimum**	**Optimum temperature**	**Stability (half life)**	**Reference**
B-glucosidase	*Acidothermus cellulolyticus*	5.0	83 °C	Inactivated at 110 °C	Sakon J. *et al.* (1996)
	Anaerocellum thermophilum	5-6	95 °C - 100 °C	40 min at 100 °C	Zverliv *et al.* (1998)
	Clostridium stercorarium	6-6.5	90 °C	Stable for several days	Bronnenmeier K *et al.* (1991)
	Clostridium thermocellum	6.6	70 °C	33% of activity remained after 50 h at 60 °C	Bergquist *et al.* (2004)
	Clostridium thermocellum	7.0	70 °C	50% of activity remained after 48 h at 60 °C	Romaniec *et al.* (1992)
	Rhodothermus marinus	7.0	95 °C	50% of activity remained after 3.5 h at 100 °C, 80% after 16 h at 90 °C	Bergquist *et al.* (2004)

	Organism	**pH optimum**	**Optimum temperature**	**Stability (half life)**	**Reference**
	Thermotoga marittima	6.0-7.5	95 °C	2 h at 95 °C	Bronnenmeier, *et al*. (1995)
	Thermotoga neapolitana	6.0	95 °C	>240 min at 100 °C	Bok *et al.* (1995)
Exoglucanase	*Clostridium stercorarium*	5-6	75 °C	3 days at 70 °C	Bronnenmeier, *et al*. (1990)

Table 2.9 : Fungal optimum condition

Fungal					
Enzymes	**Organism**	**pH optimum**	**Optimum temperature (°C)**	**Stability (half life)**	**Reference**
Endoglucanase	*Chaetomium Termphilum*	4.0	60	60 min at 60 °C	Venturi L. *et al.* (2002)
	Thermoascus aurantiacus	4.5	75	98 h at 70 °C and 41 h at 75 °C	Parry *et al.* (2002)
Exoglucanase (CBH IA)	*Talaromyces emersonii*	3.6	7.8	34 min at 80 °C	GrassikA *et al.* (2004)
Noncomplexed cellulase	*Acremonium cellulolyticus*	5.0	65	72 h at 50 °C	GrassikA *et al.* (2004)
	Trichoderma koningii	5.0	65	72 h at 50 °C	GrassikA *et al.* (2004)
	Trichoderma. reesei	5.0	65	72 h at 50 °C	GrassikA *et al.* (2004)
	Trichoderma viride	5.0	65	72h at 50 °C	GrassikA *et al.* (2004)

2.7 Acid Hydrolysis

Concentrated acids such as H_2SO_4 and HCl have been used to treat lignocellulosic materials via this acid hydrolysis method. Pretreatment with acid hydrolysis can result in improvement of the production of xylose. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive, hazardous, and thus would require reactors which are resistant to corrosion, which makes the pretreatment process to be very expensive (Priscilla *et al*, 2014). In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible. Hence, dilute-acid hydrolysis method have been successfully developed for the pretreatment process of lignocellulosic materials.

Sulfuric acid, H_2SO_4 at concentrations below 4 wt %, have been the most preferred one in such studies as it is inexpensive and much effective. Diluted H_2SO_4 will be mixed with biomass to hydrolyze the hemicellulose in order to produce xylose. Dilute acid effectively converts most of the hemicellulose into dissolved sugars. Xylose production will increase with hemicellulose removal to almost 100% via a complete hemicellulose hydrolysis. Hemicellulose is removed when H_2SO_4 is added and it will enhance the digestibility of cellulose in the oil palm frond fibre.

High temperature in the dilute acid treatment is favorable for cellulose hydrolysis in OPFF (Noah *et al.,* 2009 and Nicholas *et al.,* 2009). Recently developed dilute acid hydrolysis processes use less severe conditions and achieve higher xylose conversion yields. Achieving high xylose conversion yield is necessary to achieve favorable overall process economics because hemicellulose accounts for up to one third of the total carbohydrate in many lignocellulosic materials.Two types of dilute-acid pretreatment processes are typically used which is at a high-temperature of 160 °C and above with a continuous flow process for low solids loadings with the weight of oil palm frond fibre by the weight of reaction mixture. Another process is with a low-temperature at 160 °C and below with a batch process for higher OPFF loadings.

2.8 Factors Affecting Acid hydrolysis

2.8.1 Temperature of solution

One of the factors that should be considered particularly is the temperature of the acid and OPFF mixture as it is interrelated to acid hydrolysis process. The optimum temperature should be determined so that we could determine the optimum amount of recovery for xylose. The optimum temperature which have been determined from previous research for the production of xylose will be from 90 °C to 100 °C (Wasetorg *et al*., 2016).

2.8.2 Concentration of Acid

Different concentration of acid will be used on same amount of OPF which is 6g to test the optimum xylose production. Acid concentration ranging from 2%, 3% and 4% will be used to carry out the experiment. (Jang m wakisaka *et al.*, 2012)

2.8.3 Liquid to solid ratio of acid to oil palm frond fibre

An increase in volume of acid will cause an improvement of conversion in biomass to xylose. A hydrolysis test with constant product monitoring will be carried out. By maintaining a consistent biomass loading, it is expected to have a higher xylose productivity (quantity of sugar produced per quantity of acid) would be achieved since mixing will not be significantly affected by overwhelming solids concentrations. (Zakaria *et al.*, 2014).

2.9 Previous work on xylose recovery from oil palm empty fruit bunch (EFB)

The main objective of the previous experiment was to produce sugar (xylose and glucose) using enzymatic hydrolysis. To achieve the objective, the research was conducted to study the effect of pH, temperature and enzyme concentration. Akaline pretreatment prior to hydrolysis was done so that cellulose can be easily accessible by the enzyme. Then, enzymatic hydrolysis was performed in a temperature range of 45 °C, 55 °C, 65 °C, pH of solution between pH 6, pH7, pH8 and different enzyme concentration of 2U/mL: 3g, 4 U/mL:3g,6U/mL:3g for 48h with stirring. A commercial enzyme, xylanase derived from thermophilic fungi, *Thermomyces lanuginosus* was used in the research.

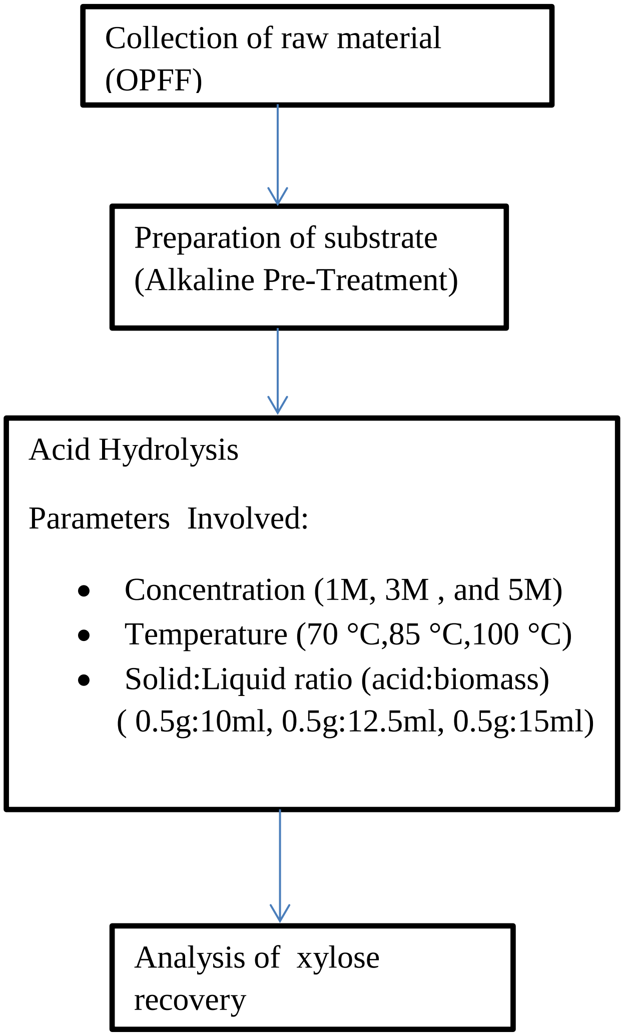
At the end of the research, the optimized xylose was obtained at temperature 65 °C, pH 6 and enzyme concentration 6 U/mL which produces 6g/L of xylose. Before optimization, the xylose production was only 1.98 g/L. In conclusion, the optimum condition for xylanase enzyme to recover xylose is at temperature at temperature 65 °C, pH 6 and enzyme concentration 6 U/mL.

CHAPTER 3

MATERIALS AND METHODS

3.0 Experimental Sequence

Figure 3.0 : Overall methodology for the production of xylose from oil palm frond fibre and the analysis involved.



3.1 Chemicals and Reagents

Hydrochloric acid, sodium hydroxide, 3,5-dinitrosalicyclic acid, Potassium Sodium Tartarate tetrahydrate, and xylose assay reagents were obtained from chemical engineering and natural resources laboratory of University Malaysia Pahang. The chemicals were sealed and stored in a container at room temperature.

3.2 Sample Preparation

The fresh oil palm frond fibres were collected from a local palm oil mill. All biomass samples were sun-dried and then cut by milling cutters into 0.5mm. (Zakaria *et al.*, 2014).

Figure 3.1: Fresh oil palm frond fibre

3.3 Dinitrosalicylic Acid (DNS) Assay

Firstly, a 56.25g of potassium sodium tartarate tetrahydrate was added inside a beaker. Then, 3g of sodium hydroxide and 93.75ml of distilled water were added together and dissolved by heating gently. When the solution is clear, 1.875g of 3,5- dinitrosalicylic acid (DNS) was added slowly. The solution was let cool to room temperature and again 93.75ml of distilled water was added to the solution.

3.4 Alkaline Pretreatment

Oil palm frond fibre (OPFF) was grinded into 0.5mm of size. Then, a 50g of OPFF was treated with 1L of sodium hydroxide solution. Then, the sample was let in water bath at 100 °C for 2 hours and the sample was let to cool down. Then, the sample was rinsed with distilled water and in order to have a neutralised sample, the oil palm frond fibre (OPFF) was treated with nitric acid and then rinsed with distilled water for 3 to 5 times again. Next, OPFF was filtered from the distilled water and let to dry in oven at a temperature of 60 °C for 24hours. (Zakaria *et al.*, 2014).

Figure 3.2 : OPFF before alkaline pretreatment

Figure 3.3 : OPFF after alkaline pretreatment

3.5 Acid Hydrolysis

After the alkaline pretreatment method, the dried oil palm frond fibre (OPFF) sample of 0.5g was hydrolysed using 10ml of 1M HCl, 12.5ml of 1M HCl and 15ml of 1M HCl for 2 hours in 70 °C of water bath. The temperature of the reaction was monitored throughout the reaction duration. The reaction was performed under atmospheric pressure and the experiment was repeated with 85 °C, and 100 °C with an acid concentration of 3M and 5M. After the acid hydrolysis reaction, the test tube was immersed in water and the hydrozylate was separated into the solid and liquid phases. Then, the xylose content in hydrozylate was determined using UV-Vis spectrophotometer with the aid of DNS reagent.

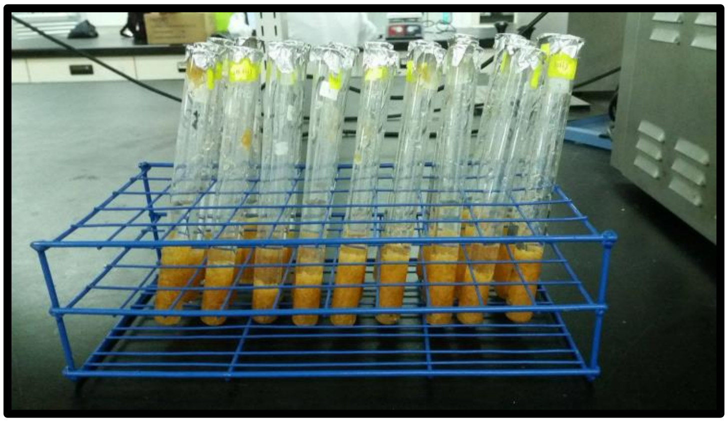


Figure 3.4 : Prepared samples for acid hydrolysis process

3.6 Analysis Methods

3.6.1 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed to determine the thermal properties of the oil palm fronds fibers for top, middle and bottom part. The thermal stability data were collected on a Perkisn Elmer TGA 7 thermogravimetric analyzer under linear temperature conditions. The temperature was swept from 30 °C to 600 °C for samples of 10-15 mg placed in an aluminium pan at a heating rate of 10 °C/min under nitrogen atmosphere.

Figure 3.5 : Thermogravimetric analysis

3.6.2 Bomb Calorimeter (Heat of Reaction Determination)

Sample was weighed (0.5 g) using analytical balance and filled in the combustion capsule. Ten centimetres of fuse wire was cut and attached to the cap downwards not touching the combustion capsule. Then, the bomb was filled with oxygen gas. Then, the combustion bomb was lowered into water. The ignition lead wires were attached into the terminal sockets on the bomb calorimeter. The cover was placed vertically on the jacket with the thermo and the stirrer was turned on. Readings were taken at one minute intervals until constant.

Figure 3.6 : Bomb Calorimeter

3.6.3 Fourier transform infra-red spectrometry (FTIR)

The presence of any changes in functional groups of the samples during the treatment were measured by FT-IR Spectroscopy. The pellets were prepared by mixing approximately 5 mg of particles samples with 95 mg of finely ground KBr before pressing it into a transparent pellet. Spectra were viewed using a Nicolet infrared spectrophotometer (Avatar 360 FT-IR E.S.P) machine. The spectra produced are transmittance mode between wavenumbers of 4000 cm-1 and 500cm-1.

Figure 3.7 : Fourier transform infra-red spectrometry

3.6.4 UV – Vis Spectrophotometer

A UV–Vis spectrophotometer is an optical device that can determine the concentration of a compound or particles in a solution or suspension. Light of a pre-selected wavelength with 575nm was shone through a chamber that houses the sample. The sample particles, absorbed some of the light. The amount of light which was absorbed increases with increasing amount of xylose in the DNS reagent. The absorbance of light was described as the optical density of the sample solution or suspension.

Firstly, standard samples were prepared in order to obtain a graph of absorbance versus xylose concentration which can be compared with the xylose obtained from the experiment. Xylose solution at the concentration of 0.0 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, and 1.6mg/mL were prepared. Then, 1.5ml of DNS reagent was added to 1.5ml of xylose solution in test tubes and was covered with an aluminium foil. The test tubes were placed in a boiling water bath for exactly 10 minutes. After being let to cool down to room temperature, 0.5ml of potassium sodium tartrate was pipetted into each tube and was mixed properly.

Then, finally the samples were transferred into cuvettes to measure the absorbance at 575nm using UV-Vis spectrophotometer. The same method was repeated during the analysis of xylose after acid hydrolysis process.

Figure 3.8 : UV – Vis Spectrophotometer

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Overview

This chapter details out the results and discussions of the recovery of xylose. The pressed oil palm frond fibre characterization will be discussed using established techniques such as Scanning Electron Microscopy (SEM), Thermogravimetric Analyzer (TGA), Fourier transform infra-red spectrometry (FTIR), Bomb Calorimeter, Spectrophotometer (Optical Density).

4.1 Thermogravimetric Analyzer

Thermogravimetry (TG) analysis shows thermal degradation of untreated, alkaline pretreated and after acid hydrolysis oil palm frond fibre. Thermal degradation is demonstrated by decrease in residual weight and increase in temperature differences due to exothermic combustion reaction (Harun et al., 2013). In general, the thermal decomposition of these fibres consists of four phases. The first phase was moisture evaporation, then decomposition of lignocellulosic components of hemicelluloses, followed by cellulose, lignin and lastly their ash. During the analysis of untreated oil palm frond fibre, a slight weight drop in the initial weight loss is due to moisture evaporation (Yang et al., 2012).

The curve shows three major steps during sample degradation. In the figure 4.1, the first one, which is observed around 25 °C and 220 °C underwent dehydration process and tightly bound water molecule was released. The degradation is as much of 8%. This might also due to the loss of volatile extractives, with less volatile extractives tending to migrate to the surface of the fibre. The migration of volatile extractives was due to the movement of water from interior of fibre to the surface of fibre since the water on fibre surface was evaporated (Yang et al., 2012).

The second one related to the degradation of hemicelluloses at around 270 °C to 346 °C, which are present in the lignin samples. The degradation is as much 52%. This might be due to the increased temperature during grinding process of freshly dried oil palm frond fibre, which caused chemical changes in their hemicelluloses components due to cellular breakdown.

The third one will be relating to degradation of lignin at temperature 345 °C to 443 °C as much as 30%. It is the most difficult to decompose compared to hemicelluloses and cellulose. Although the decomposition of lignin had started at 345 °C, it decomposes slowly and extends its temperature as high as 900 °C to complete its decomposition. This is contributed by lignin which is very tough component and known as the compound that gives rigidity to the plant materials. It is responsible for providing stiffness to the cell wall and also serves to bond individual cells together in the middle lamella region. Thus, higher lignin content resulted in increase in stiffness of the plant materials.

Peak variation (DTG curve) is used to determine activation energy of each oil palm frond fibre (OPFF) decomposition reaction. The highest peak shows the highest degradation of cellulosic component. It can be seen in second graph that the average maximum mass loss rates for the first phase is 1.005 wt%/min at 215 °C. Meanwhile, for the second phase, about 1.004 wt%/min at 270 °C and for the third phase about 1.025 wt%/min at 382 °C were found for the mass loss rate in the untreated oil palm frond fibre.

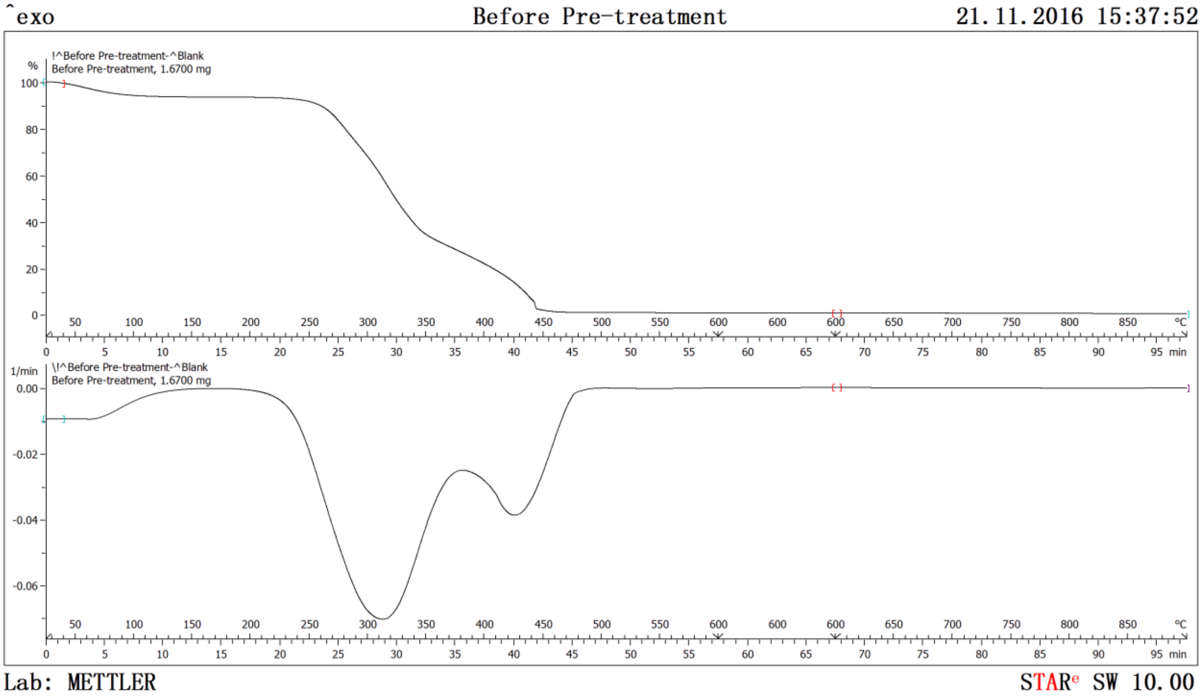


Figure 4.1 : Graph of TGA before alkaline pretreatment of OPFF

For alkaline pretreated oil palm frond fibre (OPFF) in figure 4.2, it is observed that around 25 °C and 250 °C underwent dehydration process and tightly bound water molecule was released. The degradation is around 8%. The second one which is the degradation of hemicelluloses at around 270 °C to 365 °C, which are present in the lignin samples is as much as 26%. The third one will be relating to degradation of lignin at temperature 365 °C to 460 °C which is 38%.

The highest peak shows the highest degradation of cellulosic component. It can be seen in second graph that the average maximum mass loss rates for the first phase is 1.007 wt%/min at 250 °C. Meanwhile, for the second phase, about 1.087 wt%/min at 340 °C and for the third phase about 1.05 wt%/min at 435 °C were found for the mass loss rate in the untreated oil palm frond fibre.

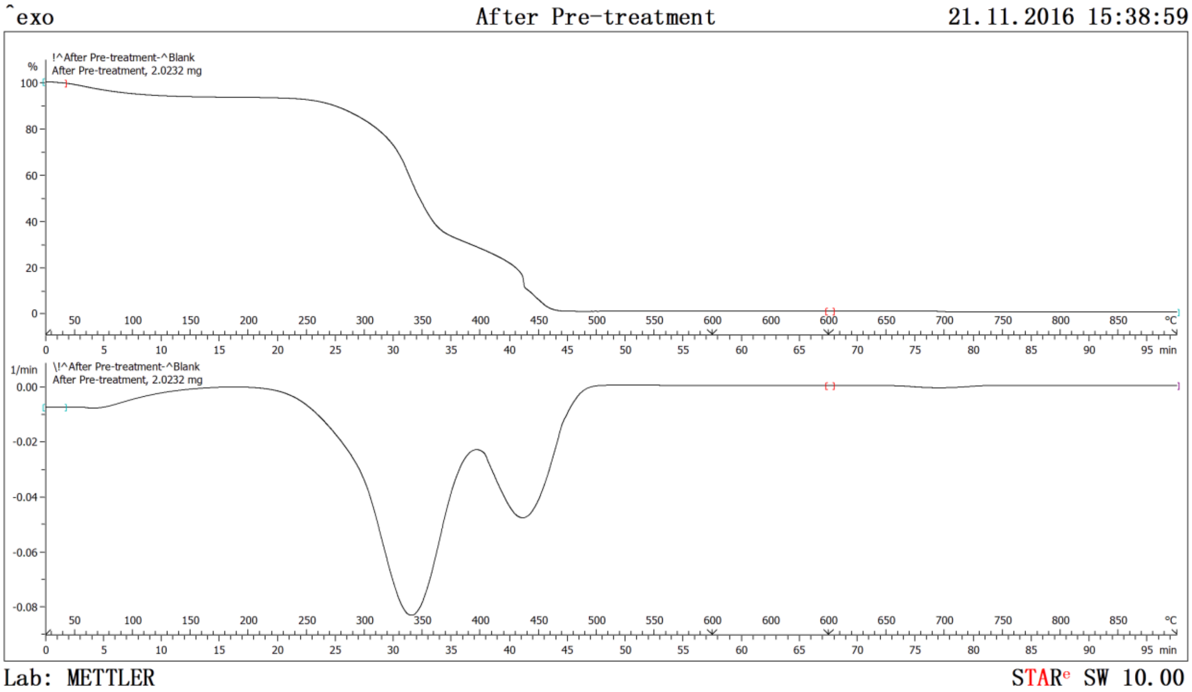


Figure 4.2 : Graph of TGA after alkaline pretreatment of OPFF

After conducting acid hydrolysis for OPFF, in figure 4.3, it is observed that around 25 °C and 240 °C underwent dehydration process and tightly bound water molecule was released. The degradation is as much as 1.009%. The second one which is the degradation of hemicelluloses at around 290 °C to 343 °C, which are present in the lignin samples which shows degradation of 18%. The third one will be relating to degradation of lignin at temperature 343 °C to 570 °C as much as 30%.

The highest peak shows the highest degradation of cellulosic component. It can be seen in second graph that the average maximum mass loss rates for the first phase is 1.009 wt%/min at 62 °C. Meanwhile, for the second phase, about 1.12 wt%/min at 330 °C and for the third phase about 1.10 wt%/min at 344 °C were found for the mass loss rate in the untreated oil palm frond fibre.

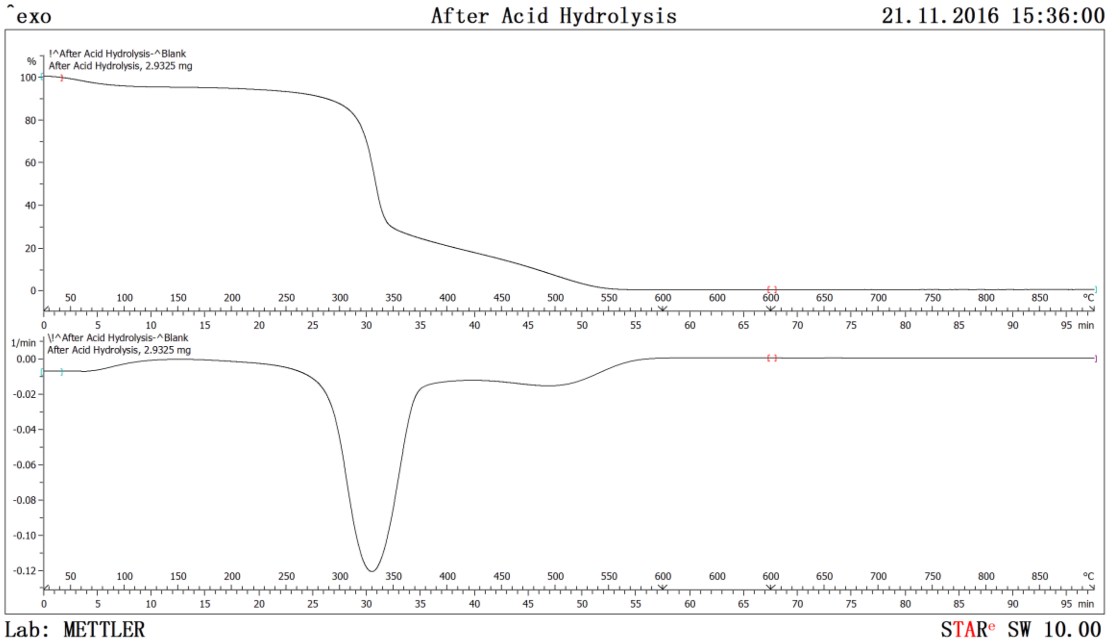


Figure 4.3 : Graph of TGA after acid hydrolysis of OPFF

From the three figures, we can conclude that the untreated oil palm frond fibre (OPFF) has lower residual weight loss compared to alkaline pretreated and acid hydrolysis oil palm frond fibre (OPFF). This is because the alkaline pretreatment and acid hydrolysis were successful in removing the hemicelluloses and some part of the less-stable lignin components. The minimal weight loss is also contributed by lower molecular components degradation of OPFF (Wan Razali et al., 2012). It is generally assumed that the temperature shift is due to the catalytic effect of the ash and that this shift leads to a single DTG peak for both cellulose and hemicellulose for high ash biomass. However, the hump apparent in the DTG curve for low ash biomass is indicative of hemicellulose and the actual low peak is due to the decomposition of cellulose.

4.2 Bomb Calorimeter

The objective of this experiment is to determine the heat of combustion and to compare the heat of combustion between samples before alkaline preteratment, after alkaline pretreatment and after acid hydrolysis. The bomb calorimeter is assumed to be adiabatic, where no heat transferred out from the calorimeter and no heat loss to the surrounding, all the heat liberated by the reaction is remained in the calorimeter. The heat released in the combustion reaction is assumed to be absorbed by the water and the other parts of the calorimeter, thereby causing the temperature of the calorimeter to rise.

Direct combustion is known as one of the conventional way to utilize oil palm waste. Combustion is capable to release hot gases up to 1000 °C. Palm oil in Malaysia are coping up their electricity and steam demand for palm oil processing by burning these wastes. Oil palm frond fibers and Palm kernel shells are generally used to fuel the power plants because of its low moisture content.

Gasification is a process that releases combustible gas mixture from biomass. The reaction occurs at temperature ranging 800 °C to 1800 °C, under relatively lower amount of oxidant to the required for the stoichiometric combustion. Through gasification, biomass is broken down into hydrogen, carbon monoxide, carbon dioxide, nitrogen and methane. These mixtures are known as syngas which can be used for heat and electric generation purpose.

Pyrolysis is thermal degradation around 400 °C to 600 °C of organic matter at ambient pressure that occurs in the absence of oxygen. The process yield products such as fuel gas, bio-oil and charcoal. Through discussion earlier, thermochemical conversion such as combustion, gasification and pyrolysis have many benefits for the utilization of oil palm biomass waste.

Calculate the H_g values for each of the samples.

From equation, the heat of combustion of sample,

$$H_g = \frac{tW - e_1 - e_2 - e_3}{m}$$

where

t = temperature rise/different

W = 2409.26 cal / °C

e_1 = correction in calories for heat of formation of nitric acid (HNO_3)

e_2 = correction in calories for heat of formation of sulphuric acid

e_3 = correction in calories for heat of combustion of fuse wire

= 2.3 x centimeters of fuse wire consumed in firing

m = mass of sample

Sample 1 (Before Pretreatment):

$$H_g = \frac{(32.3 - 30.0)(2409.26) - 17.02}{1.0}$$

= 5524.278 cal/g

= 5.524 kcal/g

Sample 2 (After Pretreatment):

$$H_g = \frac{(32.2 - 30.0)(2409.26) - 15.18}{1.0}$$

= 5285.192 cal/g

= 5.285 kcal/g

Sample 3 (After acidic hydrolysis):

$$H_g = \frac{(32.0 - 30.0)(2409.26) - 13.57}{1.0}$$

= 4804.95 cal/g

= 4.805 kcal/g

From the result obtained, we can see that the heat of combustion of oil palm frond after acidic hydrolysis is lower than the heat of combustion of oil palm frond fibre before and after pretreatment. This is because the rate of reaction for the combustion is highly affected by surface area of oil palm frond fibre. Before pretreatment process, the fronds are having strong cellulose-hemicellulose lignin network which have smaller surface area which would need more amount of heat to burn the sample.

After acid hydrolysis process, the surface area increases as the structure will be disrupted badly due to the reaction between oil palm frond fibre and acidic solution with the aid of hot water. Thus, lower amount of heat is required to burn the sample. Meanwhile, oil palm frond fibre before pretreatment have higher heat of combustion value compared to after pretreatment process. This is because, the structure of oil palm frond fibre would be mildly broken due to the alkaline treatment which was aimed to make the fibre become more porous to extract more xylose during the acid hydrolysis pretreatment.

4.3 Fourier Transform Infra-red Spectroscopy (FTIR)

FTIR spectroscopy is an analytical tool for qualitatively determine the chemical changes and functional group in the oil palm frond fibre (OPFF) surface before pretreatment, after alkaline pretreatment and after acid hydrolysis process. Table 4.1 shows the functional group of chemical substances and its specific wavenumber (Li et al.,2008).

Table 4.1 : Functional group according to wavenumber (Li et al.,2008)

Wavenumber (cm^{-1})	Functional Group
1030 – 1155	C- O
1550 - 1650	NH_2
600 - 400	Aromatic ring
2500 – 3500	O-H
2880 – 2500	C- H
3300 - 3500	N-H

Figure 4.4 shows FTIR spectrum for fresh untreated oil palm frond fibre (OPFF) sample. The first peak at 3330.70 cm^{-1} indicates the strong bonded functional group of N-H stretching absorption. Prominent C-C stretching was seen around the range of peak between 2217.36 to 2100.20 cm^{-1}. Next, the peak at 1732.60 cm^{-1} represents strong C-O stretching functional group. The peaks at 1593.36 until 1423.76 cm^{-1} indicates the presence of heterocyclic compounds. 1371.91 cm^{-1} shows bonded CH deformation mode. The peak at 1031.50 cm^{-1} shows C-O functional group while 419.67 cm^{-1} until 518.20 cm^{-1} indicates that strong aromatic ring stretching in cellulose. Fingerprint region occurs towards the end. Fingerprint region is the region consists of absorptions due to all other single bonds (except H-Z), making it often a complex region that is very difficult to analyze.

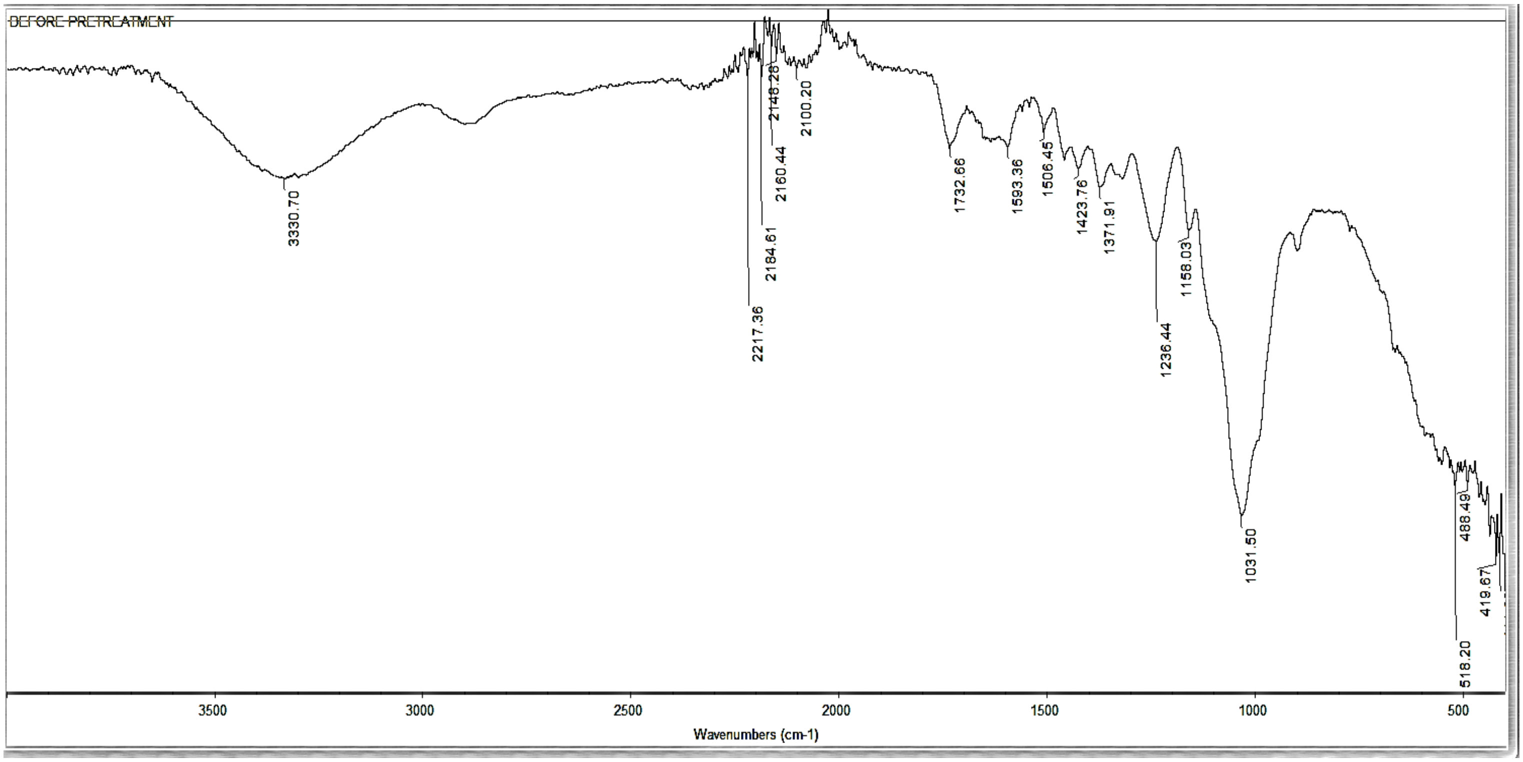


Figure 4.4 : Graph of transmittance vs wavenumber before pretreatment of OPFF (cm^{-1})

Figure 4.5 shows FTIR spectrum of alkaline pretreated OPFF sample. The peak at the range of 3752.40 cm^{-1} until 3629.84 cm^{-1} indicates the strong bonded functional group of N-H. Besides the range of 3320.81 cm^{-1} until 2890.76 cm^{-1} indicates O-H stretching absorption. The peak at 2109.83 cm^{-1} shows C-C bond absorption. Prominent NH_2 stretching was seen around peak 1590.98 cm$^{-1.}$ The peak at 1500.84 cm^{-1} until 1420.57 cm^{-1} shows the presence of heterocyclic compounds meanwhile the peak at the range of 1319.56 cm^{-1} until 1155.99 cm^{-1} shows the presence of C-H bond absorption. Besides, the peak at 1026.76 cm^{-1} shows the presence of C-O group, the peak at 896.32cm^{-1} shows the presence of inorganic phosphate compound and the peak at 434.02 cm^{-1} shows the presence of minimal amount of aromatic ring.

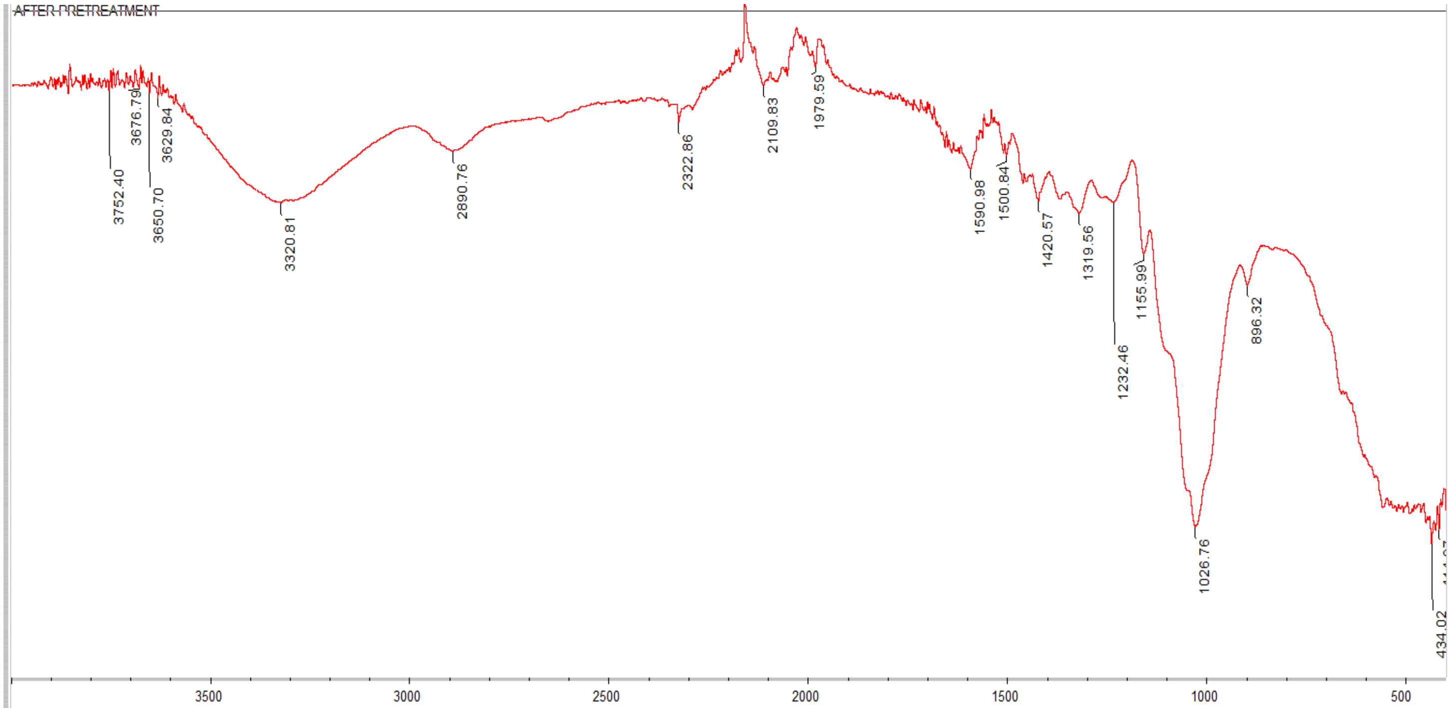


Figure 4.5 : Graph of transmittance vs wavenumber after alkaline pretreatment of OPFF (cm^{-1})

Figure 4.6 shows FTIR spectrum after acidic hydrolysis sample. The first peak at 3331.34cm^{-1} indicates the strong bonded functional group of N-H stretching absorption. The second peak at 2895.10cm^{-1} shows C-H stretching absorption. Prominent NH_2 stretching was seen around peak at 1593.20cm^{-1} .Then, the peak in the range of 1501.29 cm^{-1} until 1421.30 cm^{-1} shows the presence of heterocyclic compounds meanwhile the peak range between 1315.08 cm^{-1} until 1027.73 cm^{-1} shows its CH_2 vibration. Besides, the peak in the range between 895.65 cm^{-1} until 557.63 cm^{-1} shows the presence of inorganic phosphate compound. The last peak which is 433.78 cm^{-1} is an aromatic ring.

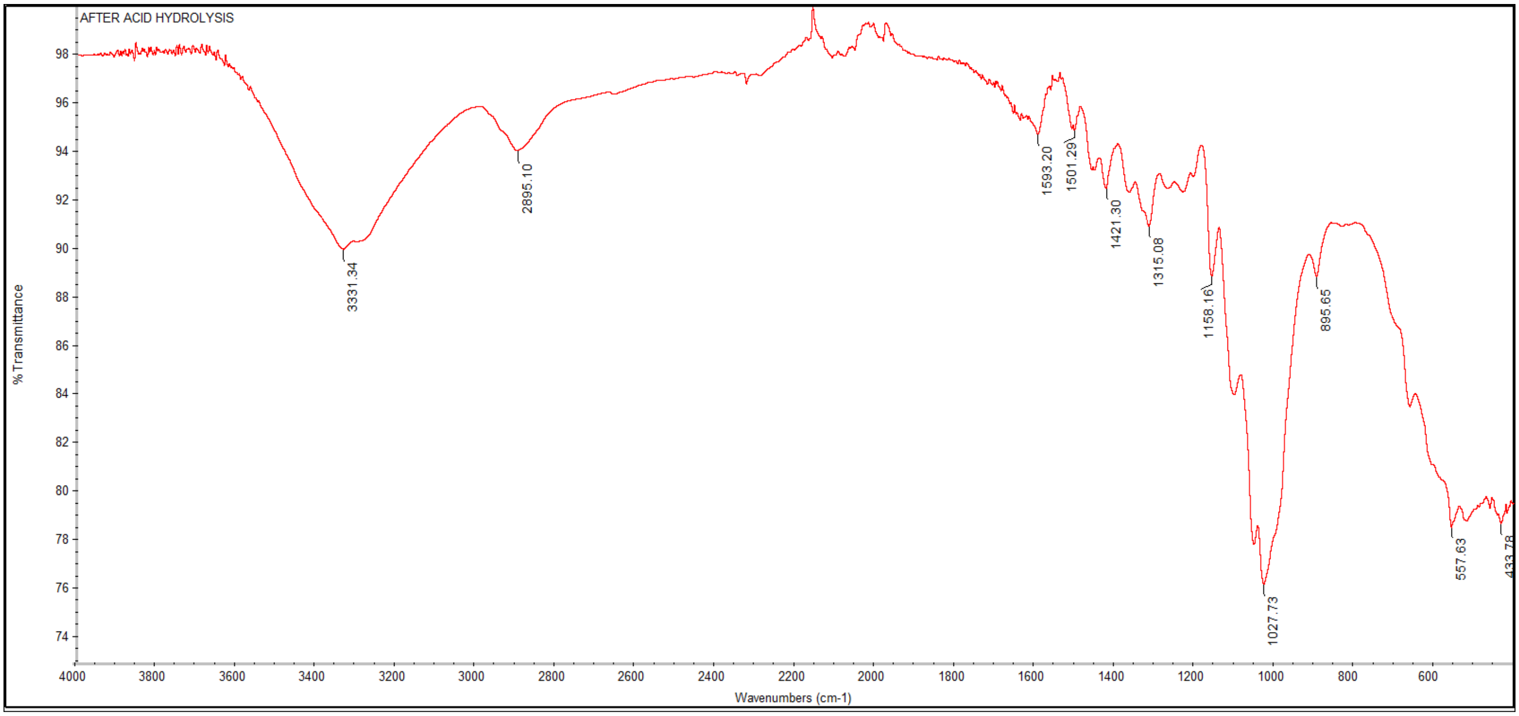


Figure 4.6 : Graph of transmittance vs wavenumber after acid hydrolysis of OPFF (cm^{-1})

The spectra of all samples indicated that the most significant changes of the wavenumber were those between 2850 cm^{-1} and 3000 cm^{-1} which is associated with C-H stretching of lignin, hemicelluloses and cellulose all decreased after pretreatment of oil palm frond fibre and after acidic hydrolysis of oil palm frond fibre. Based on these, we can conclude that some of the functional group were affected by the physical treatment and chemical addition and stronger changes occurs when both methods were applied (Ray and Sarkar, 2001). Another significant changes of peaks with 1900 cm^{-1} and 2300 cm^{-1} were also noticed but qualitative analyses used in present study unable to quantify the differences in the samples.

4.4 UV – Vis Spectrophotometer

Calibration curve helps to have a reasonable estimated ranges of concentrations of sample so that we can obtain the exact value of concentration for the tested samples from acid hydrolysis process. Even with such an estimate it is good to prepare samples with a range of dilutions, in case a sample is so concentrated that its absorbance readings are out of range. Table 4.2 shows the result for standard xylose solution which was prepared to give out a range of absorbance reading according to the different values of concentration for xylose. Meanwhile, figure 4.7 shows the colour changes occurred in the standard xylose solution after a 1.5ml of DNS assay was pipetted into each of the test tubes.

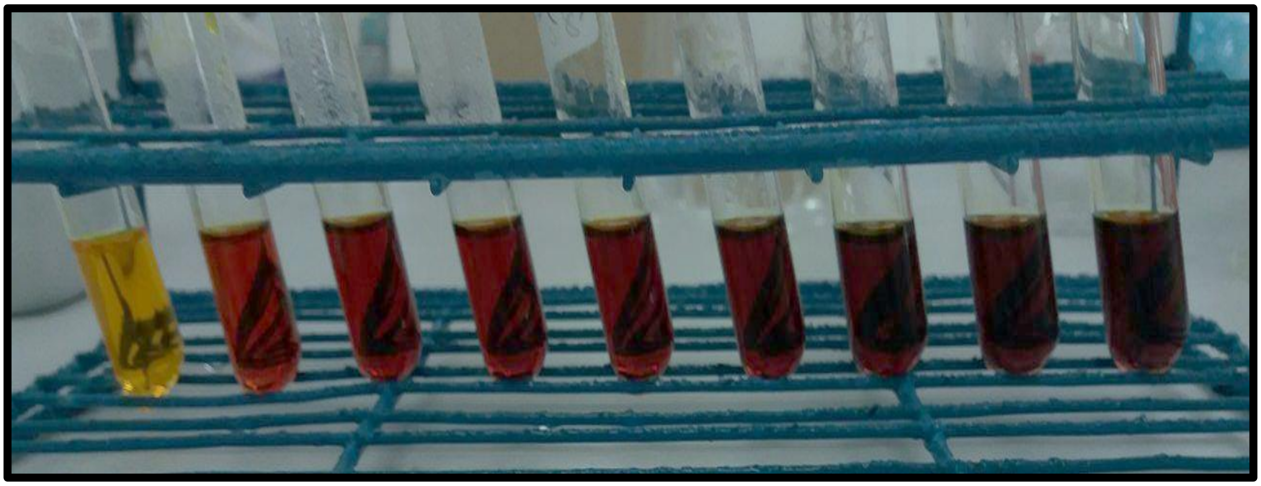


Figure 4.7 : Standard xylose solution with DNS assay after the heating process

Table 4.2 : Absorbance of standard xylose solution for different concentrations

Concentration (mg/mL)	Absorbance Reading	Average Absorbance Reading
0	0.1271	0.1267
	0.1266	
	0.1264	
0.2	0.4706	0.4702
	0.4701	
	0.4698	
0.4	0.8906	0.8905
	0.8903	
	0.8906	
0.6	0.9058	0.9057
	0.9058	
	0.9056	
0.8	1.1848	1.1848
	1.1849	
	1.1847	
1	1.3345	1.3348
	1.335	
	1.3349	
1.2	1.4582	1.4582
	1.4582	
	1.4582	
1.4	1.5522	1.5528
	1.5537	
	1.5525	
1.6	1.7039	1.7033
	1.7038	
	1.7021	

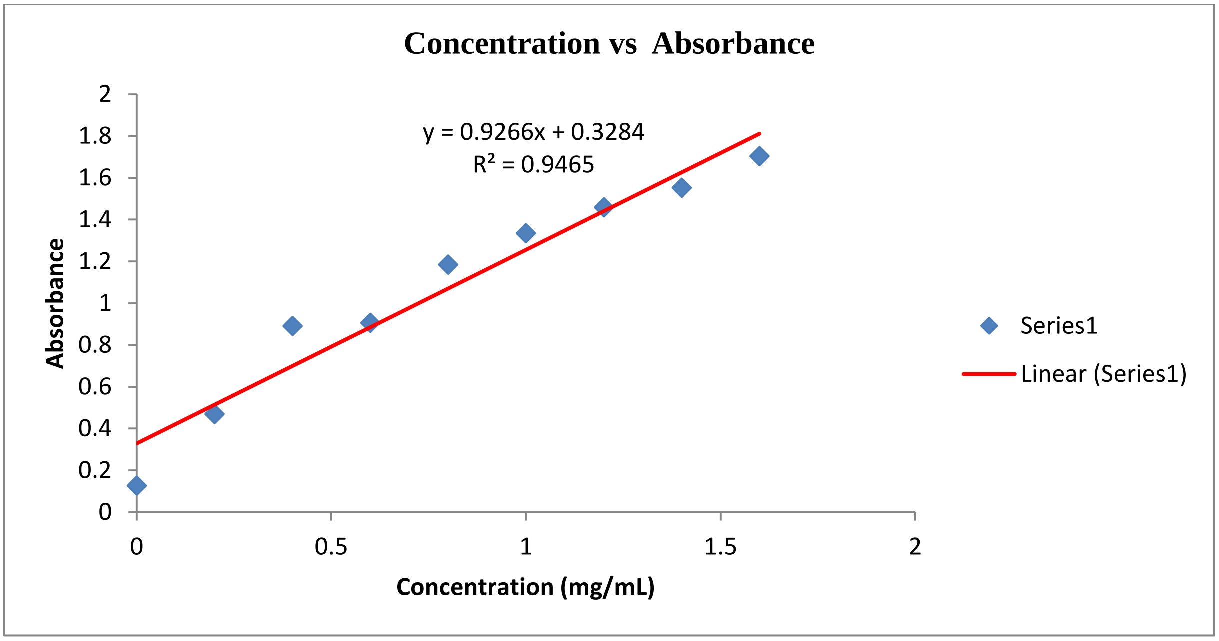


Figure 4.8 : Calibration curve of concentration versus absorbance of standard xylose solutions

Based on the readings obtained from table 4.2, it is found that the value of absorbance for the standard solution was increasing gradually as the concentration of xylose in the sample increases. In the figure 4.8, it is found that the R^2 value for this standard solution is 0.9465 which is slightly less that the value of 1.0000. This might be due to few errors or disturbances present during the standard sample preparation which affected the reading of absorbance obtained. Disturbances due to the presence of impurities in test tubes or other equipment such as syringe, glass rod, and beaker caused the values of absorbance to vary a little from the actual reading that we supposed to get. However, the value of $R^{2 \text{ is}}$ still accepted because it is nearer to the value of 1.0000.

Table 4.3 : Average Absorbance and concentration of xylose (mg/mL) values when the concentration of HCl was 1 M

Concentration Of HCl (M)	Temperature (°C)	Solid : Liquid Ratio	Average Absorbance	Concentration of xylose (mg/mL)
1	100	0.5 : 10	0.1852	0.0341
1	100	0.5 : 12.5	0.5253	0.2262
1	100	0.5 : 15	0.1920	0.0380
1	85	0.5 : 10	0.4854	0.2072
1	85	0.5 : 12.5	0.0680	-
1	85	0.5 : 15	0.1606	0.0197
1	70	0.5 : 10	0.1058	-
1	70	0.5 : 12.5	0.3205	0.1128
1	70	0.5 : 15	0.0550	-

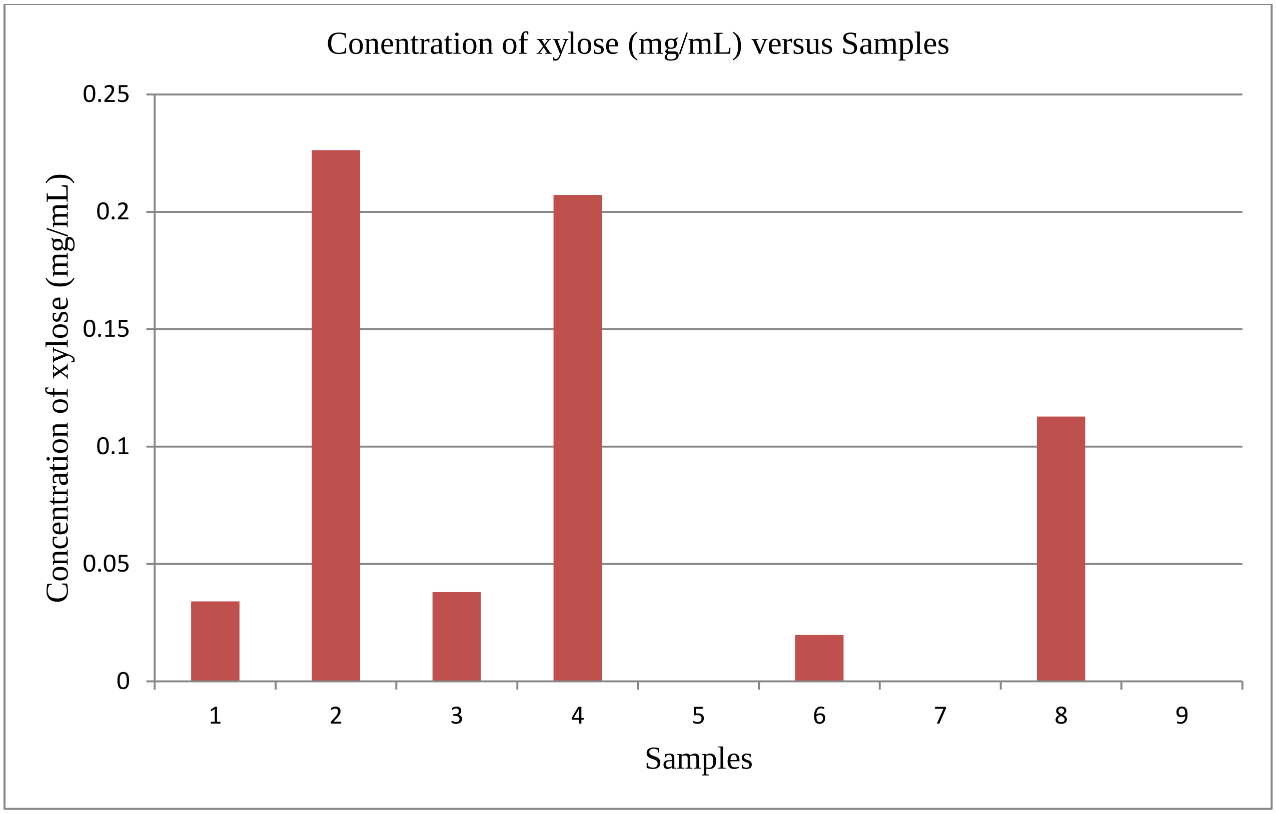


Figure 4.9 : Concentration of xylose (mg/mL) versus samples with a HCl concentration of 1 M

Based on the analysis from Figure 4.9, it is found that the sample 2 with a concentration of 1M, temperature at 100 °C and liquid to solid ratio of 0.5g:12.5ml have the highest concentration of xylose which is 0.2262 mg/mL. Meanwhile, sample 5, 7 and 9 shows zero detection for xylose is due to the presence of impurities in samples while the experiment was conducted. Besides, sample 6 shows the second lowest concentration of xylose in a condition of 1 M and 0.5g OPFF: 15 mL of HCl at 85 °C. This is because a lower concentration of acid which is 1 M requires higher temperature about 100 °C to have higher yield of xylose meanwhile 15 mL of acid solution is suitable to have enough molecules to react with oil palm frond fibre (OPFF) and produce more xylose at this condition.

Table 4.4 : Average absorbance and Concentration of xylose values when the concentration of HCl was 3 M

Concentration of HCl (M)	Temperature (°C)	Solid : Liquid Ratio	Average Absorbance	Concentration of xylose (mg/mL)
3	100	0.5 : 10	0.9102	0.6032
3	100	0.5 : 12.5	0.0745	-
3	100	0.5 : 15	0.0171	-
3	85	0.5 : 10	0.0270	-
3	85	0.5 : 12.5	0.1265	-
3	85	0.5 : 15	0.6182	0.2704
3	70	0.5 : 10	0.3670	0.1399
3	70	0.5 : 12.5	0.1596	0.0192
3	70	0.5 : 15	0.5414	0.2339

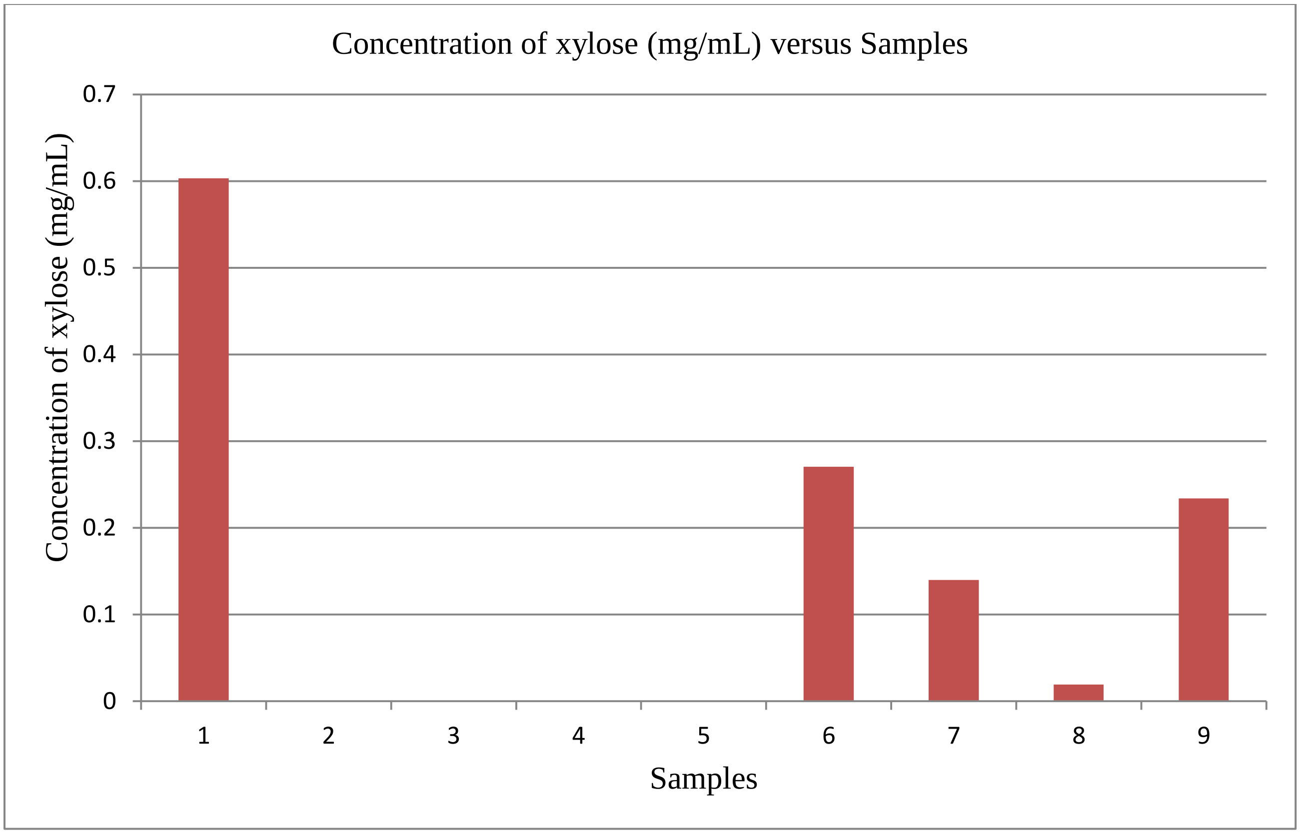


Figure 4.10 : Concentration of xylose (mg/mL) versus samples with a HCl concentration of 3 M

Based on the analysis from Figure 4.10, it is found that the sample 1 with a concentration of 3 M, temperature at 100 °C and liquid to solid ratio of 0.5 g:10 mL have the highest concentration of xylose which is 0.6032 mg/mL. Besides, sample 8 with a concentration of 3 M, temperature at 70 °C and solid to liquid ratio of 0.5 g:12.5 mL have the second lowest concentration of xylose which is 0.0192 mg/mL. Meanwhile, sample 2, 3, 4 and 5 are giving a xylose concentration of zero as the actual concentration of the samples lies below the lower end of standard curve. This is mainly due to the presence of unknown impurities while the experiment was conducted.

Table 4.5 : Average absorbance and Concentration of xylose values when the concentration of HCl was 5 M

Concentration (M)	Temperature (°C)	Solid : Liquid Ratio	Average Absorbance	Concentration of Xylose (mg/mL)
5	100	0.5 : 10	0.5247	0.2259
5	100	0.5 : 12.5	1.1495	0.7747
5	100	0.5 : 15	0.5341	0.2304
5	85	0.5 : 10	0.7630	0.3393
5	85	0.5 : 12.5	0.8225	0.3676
5	85	0.5 : 15	0.7366	0.3268
5	70	0.5 : 10	0.6465	0.2839
5	70	0.5 : 12.5	0.9430	0.6267
5	70	0.5 : 15	1.3461	1.0183

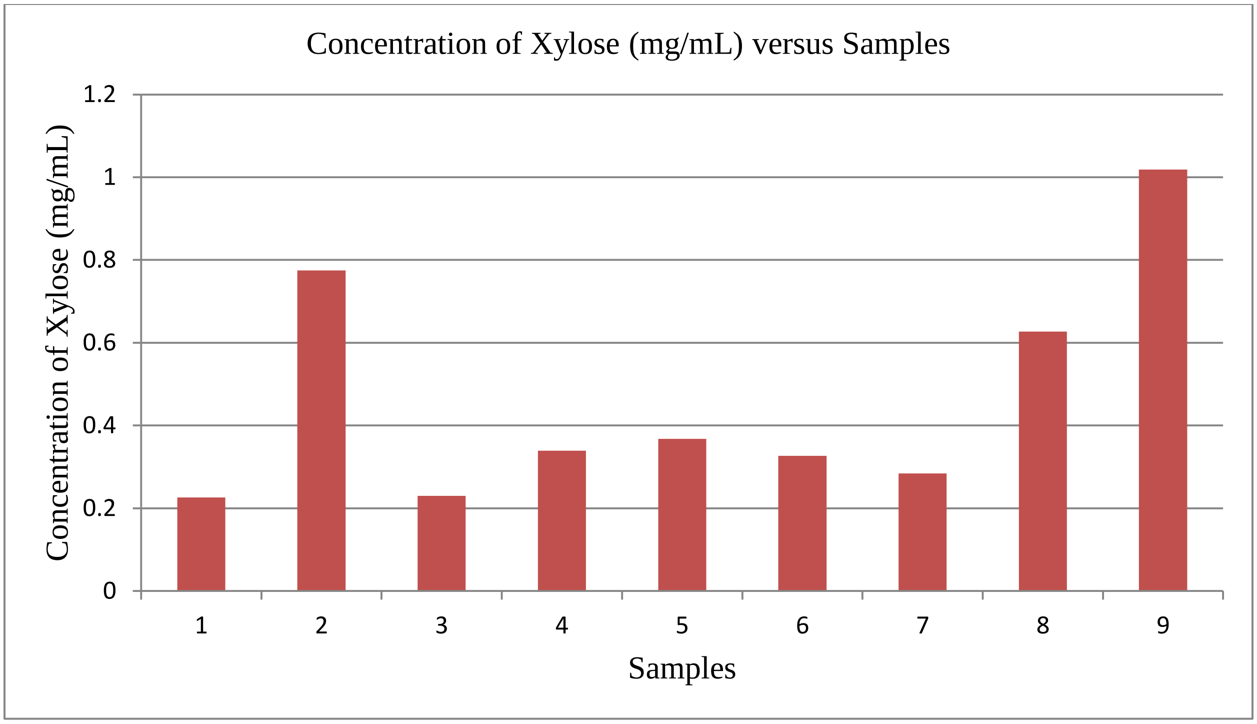


Figure 4.11 : Concentration of xylose (mg/mL) versus samples with a HCl concentration of 5M

Based on the analysis from Figure 4.11, it is found that the sample 9 with a HCl concentration of 5 M, temperature at 70 °C and solid to liquid ratio of 0.5 g:15 mL have the highest concentration of xylose which is 1.0183 mg/mL. Meanwhile, sample 1 with a concentration of 5 M, temperature at 100 °C and liquid to solid ratio of 0.5 g:10 mL have the lowest concentration of xylose which is 0.2259 mg/mL. As the experiment was carried out to use an acid concentration of 5 M, the xylose was found to be the highest with low temperature at 70 °C compared with 100 °C.

This is because, a higher temperature of solution can damage the structure of OPFF and causes less amount of xylose to be released, yet the xylose yield in the first sample with acid concentration of 5 M was not too low compared with the first sample from experiments using acid concentration of 1 M and 3 M. This is mainly because, as a higher concentration of acid solution is imposed on the OPFF, immediately the solution reacts with OPFF to break the hemicellulose walls to release the xylose content. However, prolonged exposure of this concentrated acid solution on OPFF causes it to be denatured or burnt, thus the reduction in xylose can be seen clearly.

In conclusion, throughout the analysis of this experiment it is found that the best condition to produce a higher yield of xylose is at an acid concentration of 5 M, temperature at 70 °C and solid:liquid ratio of 0. 5g:15 mL.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Oil palm frond fibre (OPFF) is one of the lignocellulosic material which is rich in hemicellulose contents which can be converted to xylose which is a form of sugar. For this research, oil palm frond fibre (OPFF) is used as raw material and undergoes alkaline pretreatment with sodium hydroxide and acid hydrolysis process to produce xylose. From the objective of this study which is to recover xylose from pressed oil palm frond fibre and study the effect of acid concentration, solid to liquid ratio of oil palm frond fibre to volume of acid and temperature of the solution. The results shows the highest value of xylose recovery from OPFF was 1.0183 mg/mL at the acid concentration of 5 M , solid to liquid ratio which is 0.5 g:15 mL of oil palm frond fibre (OPFF) to volume of acid and temperature of 70 °C. The combination between these parameters have enhanced the production of xylose.

The value of xylose recovery is mainly influenced by the alkaline pretreatment because during this process the structure of oil palm frond fibre will be broken further thus, it causes the OPFF structure to be more porous and yields more xylose during the acid hydrolysis process. Effect of parameters such as acid concentration, solid to liquid ratio and temperature on xylose production from hemicelluloses has been successfully carried out in this study. Besides that, the objective of this experiment has been successfully achieved by obtaining different amount of xylose according to its respective conditions.

In conclusion, the oil palm frond fibre (OPFF) is able to be used as an alternative source in xylose production and acid concentration of 5 M, solid to liquid ratio of 0.5 g of biomass to 15 mL volume of acid and temperature 70 °C is suitable to get the optimum xylose production. Last but not the least, pretreatment should be done to increase the yield of xylose.

5.2 Recommendation

As shown in the results, xylose is possible to be recovered through acid hydrolysis on oil palm frond fibre (OPFF) biomass. As for recommendation, further study should be done to study the condition or experimental work procedure which is possible to yield high value of xylose concentration. The recommendations are:

i. The use of other acids such as sulphuric acid, nitric acid and phosphoric acid in acid hydrolysis.

ii. Increase the surface area of raw material by making the OPFF into a very fine powder.

iii. Research on other parameters such as substrate concentration, rpm rate and time.

iv. Research on different pretreatment for OPFF.

v. Research on other raw material such as sugarcane baggase, rice straw, corn cub and empty fruit bunches.

Research on different kind of raw materials, parameters and ways will provide wide opportunities to have higher production of xylose in the future.

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