PRODUCTION OF CELLULOSIC SUGARS FROM OIL PALM EMPTY FRUIT BUNCH

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE

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KUALA LUMPUR

2013

ABSTRACT

Dilute perchloric acid of up to 12% concentration has been used to digest ground biomass of oil palm empty fruit bunch (OPEFB). The method by-passes the need to chemically pretreat the substrate as the acid perchloric is able to pretreat the lignin and hemicellulose, and subsequently saccharify the cellulosic residues to glucose and other sugars. Two parameters for the dilute acid treatment, namely acid concentration and time of reaction were investigated to optimize the yield of glucose. The optimum conditions for perchloric acid hydrolysis of OPEFB with respect to different concentrations of acid and time at 100˚C were reported. Perchloric acid concentration of 12% and the time of 180 min gave the best yield of 43.7 g of total sugars per 100 g of dry OPEFB. Comparative studies under similar conditions were made for hydrochloric acid, sulphuric acid and perchloric acid. The total sugar yield for sulphuric acid and hydrochloric acid hydrolysis was 27.8 g and 26.7 g of total sugars per 100 g of dry OPEFB, respectively. Excess perchloric acid was neutralised with KOH to yield a precipitate of $KClO_4$, a salt that is easily filtered to yield the sugar-rich hydrolysate. $KClO_4$ may be recycled to perchloric acid. The method represents an option for the production of sugars from lignocellulosic biomass that may be further processed for the production of bioethanol. The final yield of ethanol was 97% based on glucose.

ABSTRAK

Asid perklorik cair sehingga kepekatan 12% telah digunakan untuk mencerna biojisim tandan kosong buah kelapa sawit. Kaedah ini memintas keperluan untuk menjalankan pra-rawat kimia ke atas substrat memandangkan asid perklorik dapat merawat lignin dan hemiselulosa, dan kemudiannya menukarkan selulosa kepada glukosa dan gula lain. Dua parameter untuk rawatan asid cair telah di kaji, iaitu kepekatan asid dan masa tindak balas untuk mengoptimumkan hasil glukosa. Keadaan optimum asid perklorik untuk menghidrolisis tandan kosong buah kelapa sawit dengan kepekatan asid dan masa pada 100˚C telah dilaporkan. Kepekatan asid perklorik sebanyak 12% dan masa selama 180 min memberikan hasil yang terbaik iaitu sebanyak 43.7 g gula untuk setiap 100 g tandan kosong buah kelapa sawit kering. Menggunakan kaedah yang sama, kajian perbandingan telah dibuat bagi asid hidroklorik, asid sulfurik dan asid perklorik. Keputusan menunjukkan hasil gula terhasil ialah 27.8 untuk asid sulfurik g dan 26.7 g untuk asid hidroklorik bagi setiap 100 g tandan kosong buah kelapa sawit kering. Asid perklorik berlebihan telah di neutralkan dengan KOH untuk menghasilkan mendakan garam $KClO_4$ yang mudah ditapis untuk menghasilkan hidrolisat yang kaya dengan gula. $KClO_4$ boleh dikitar semula kepada asid perklorik. Kaedah ini merupakan satu pilihan untuk pengeluaran gula daripada biojisim lignoselulosa yang boleh terus diproses untuk pengeluaran bioetanol. Hasil akhir etanol adalah sebanyak 97% berdasarkan glukosa.

ACKNOWLEDGMENT

First of all, I would like to express gratitude to my supervisor, Prof Zainudin Arifin and Dr Teoh Teow Chong, for encouragement, guidance, criticism and assistance in the completion of this thesis.

I also wish to thank Institute of Research Management and Consultancy (IPPP) for providing financial support (Research grant number RG019/09BIO and PV040/2011A) and MARDI for providing samples. My appreciation also goes to the Department of Chemistry, Faculty of Science, University of Malaya for support and facilities to carry out the experimental work.

Last but not least, I would like to thank to Institute of Graduate Study (IGS), my family and friends for their moral support.

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LIST OF ABBREVIATIONS

AFEX	-	Ammonia Fibre Explosion
C	-	Carbon
CH_4	-	Methane
$C_6H_{12}O_6$	-	Glucose
CO_2	-	Carbon Dioxide
EFB	-	Empty Fruit Bunch
HCl	-	Hydrochloric Acid
$HClO_4$	-	Perchloric Acid
H_2O	-	Water
H_2SO_4	-	Sulphuric Acid
K	-	Potassium
$KClO_4$	-	Potassium Perchlorate
KOH	-	Potassium Hydroxide
Mg	-	Magnesium
N	-	Nitrogen
NO_X	-	Nitrogen Oxide
O_2	-	Oxygen
OPEFB	-	Oil Palm Empty Fruit Bunch
P	-	Phosphorus
PKC	-	Palm Kernel Cake
PKS	-	Palm Kernel Shell
POME	-	Palm Oil Mill Effluent
PPF	-	Palm Press Fibre

SC	-	Sludge Cake
SO_X	-	Sulphur Oxide

LIST OF SYMBOLS

%	-	percents
˚C	-	degree Celsius
α	-	alpha
β	-	beta
µ	-	micrometre
g	-	gram
M	-	Molar
min	-	minute
mL	-	millilitre
v/v	-	volume per volume

CHAPTER 1

INTRODUCTION

1.1 Biomass

Biomass is composed of biological material of living or recently living organism and may be considered as a renewable energy source. It includes forestry and agriculture crops and residues, animal and industrial residues, and sewage and municipal solid waste. These sources can be divided into five distinct energy sources; garbage, wood, waste, landfill gases and alcohol fuels; and have long been identified as sustainable sources of renewable energy (Yan *et al*., 1997; Ong *et al*., 2011; Goh *et al*., 2010).

Biomass has gained great attention due to its high energy content that may be utilized on a sustainable basis and this energy source has been exploited around the globe. If this bio-energy supply is maintained in a sustainable way, it can contribute positively to the ecological well-being of the local and global environment. Biomass energy can be converted into a large energy source such as electricity (bio-power) and heat or to a small energy source such as a wood stove or small wood-fired furnace (Sumathi *et al*., 2008). It supplies about 9-13% of global energy use which includes both traditional uses such as cooking and heating or modern use like producing electricity and steam.

The conversion of non-edible biomass waste into fuels such ethanol, methanol, bio-oil and bio-diesel has increased significantly and could help to solve the energy shortage that has occurred in many areas worldwide. This biomass has been recognized as a major renewable energy source as it contains the most organic compounds on earth. The current high energy price has become a serious threat to mankind due to the over dependence on fossil fuel as the main source of energy for most countries and communities over the last decade. As the world economy grows, demand on energy increases and this situation has caused governments and authorities to focus on developing biomass as an alternative renewable energy resource (Ong *et al*., 2011). As an added windfall to the above effort, researchers are churning out a wider variety of by-products by the downstream processing of biomass that has now been developed into a promising future industry.

Currently, biomass can be converted to high value commodity chemicals and fuels using the appropriate technology (Rass-Hansen *et al*., 2007). Fossil fuel generates green house gas emissions (CO_2, CH_4, SO_X, NO_X) that lead to global warming and thereby climate change. 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 (McKendry, 2002). Biomass is basically carbon, hydrogen and oxygen-based. The principle of producing energy from biomass corresponds directly to photosynthesis where carbon dioxide and water are transformed to oxygen gas and glucose through input of energy from the sun:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

Biomass is a CO_2 neutral energy source based on the fact that the CO_2 released during burning is taken up by growing plants provided that the rate of harvest of wood is equal to the rate of its regrowth. In particular the use of cellulose waste as fuel does not add to the CO_2 footprint as it is recycled during plant growth thereby helping alleviate the climate change crises (Escobar *et al*., 2009).

Ethanol is a prime example of biomass alcohol fuel and it is produced from starch, sugar crops and agricultural residues. However, much of current bioethanol is produced from food portion which poses ethical concerns about competition between food and feed supplies.

Alternatively, the second generation of feedstock such as agriculture residues is expected to be a new source for bioethanol production. The annual global production of agricultural residue is predominantly produced by corn stover, wheat straw, paddy straw, sugarcane bagasse, oil palm residue and soybean shown in Figure 1.1 (USDA, 2012). In order to utilize the large amount of waste, novel technologies with improved efficiencies and reduced environmental impact are needed to be established in time (Yang *et al*., 2006). In addition, biomass conversion contributes significantly to national economic activities such as job creation.

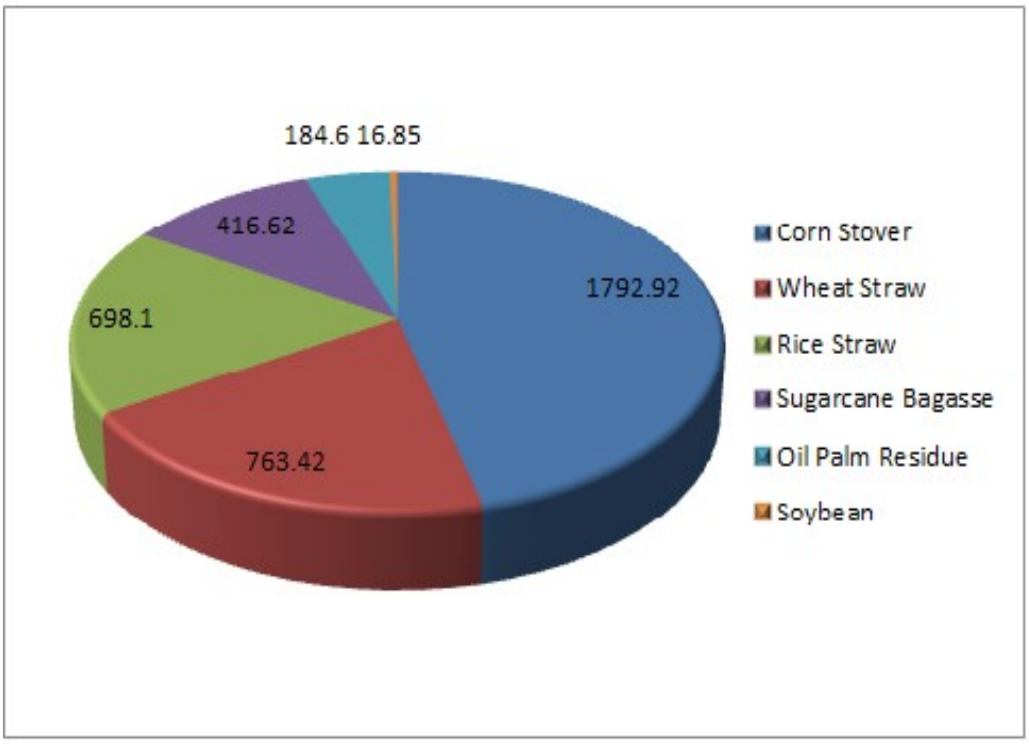


Figure 1.1: Quantities of lignocelluloses biomass in worldwide (million tons) (USDA, 2012).

1.2 Biomass from Oil Palm

Oil palm, *Elaeis guineesis*, is cultivated in all tropical areas and originates from west and central Africa (Chew and Bathia, 2008). Palm oil is an important part of the agro-industry in Malaysia, Indonesia and Thailand where these countries export over 90% of world's palm oil. Malaysia is the second largest producer of palm oil for the last 25 years with approximately 40-60% of total world palm oil production replacing Nigeria which was the chief producer before 1971 (Mongabay.com, 2007; Sumathi *et al*., 2008; Sumiani, 2006). In more than three decades, Malaysia has had the monopoly of being a global leader of this industry and provides more than 1.4 million jobs (Malaysian Palm Oil Board, 2010; Craven, 2011; Malaysia Innovation Agency, 2011).

The oil palm is a tropical palm tree which can be easily cultivated in Malaysia. Malaysia blessed with favourable weather; climatic condition like being wet and humid is suitable is for planting oil palm trees. Malaysia and Indonesia dominate in production of crude palm oil counting up to 35 million tons in 2010 due increasing demand in vegetable oil (USDA, 2010). The first commercial oil palm estate in Malaysia was set up in 1917 at Tennamaran Estate in Selangor (Malaysian Palm Oil Council, 2006; United Nations Development Program, 2006). In 1950, only about 38,000 ha of the land were used for oil palm plantation. Production of a single hectare can produce 10 times more oil than other oilseeds. The fruits from oil palm have many uses such as for extraction of edible oil and for cooking.

Oil palm biomass is one of the biggest resources of renewable energy and contributes to about RM6,379 million of energy annually. There are various forms of wastes from the mills such as empty fruit bunch (EFB), palm pressed fiber (PPF), palm kernel cake (PKC), palm kernel shell (PKS), sludge cake (SC) and palm oil mill effluent (POME) (Sumiani, 2006). The quantity of waste depends on the raw materials and only OPEFB, PPF and PKS are considered as waste and are available in large quantities. The other waste is used as food for ruminants (cattle and goats) or left to rot for soil conservation, which increases the fertility of soil, controls erosion and provides a source of nutrients for oil palm trees (Sumathi *et al*., 2008; Palm Oil Industry, 2006; Aspar, 2005). The OPEFB has traditionally been used as fertilizer or soil conditioner as incinerated ash. However, the high moisture content of OPEFB at 60% contributes to the “white smoke” problem (Sumiani, 2006). The DOE discourages the incineration of OPEFB due to environment pollution.

In the year 2011, Malaysia generated 7.9, 13.2 and 23.8 million tons of fiber shell and OPEFBs, respectively (Malaysian Palm Oil Board, 2011). A fresh fruit bunch produces leaves behind 20-30% of OPEFB and the bulky nature of the OPEFB causes a high land-fill disposal cost which needs to be burnt into ash. This emits CO_2, CO, NO_2 and causes air pollution (Prasertsan and Prasertsan, 1996). Oil palm fiber especially OPEFB is rich in cellulose and most research papers show that OPEFB has been used to produce glucose and xylose successfully (Lim *et al*., 1997). OPEFB consists of 42% C, 0.8% N, 0.06% P, and 2.4% K and 0.2% Mg (Krause, 1994). OPEFB, a by-product of the palm oil industry obtained after pressing the oil from the fruit bunch, is composed mainly of cellulose (41.3–46.5%), hemicellulose (25.3–33.8%) and lignin (27.6–32.5%) (Ariffin *et al*., 2008; Hamzah *et al*., 2011; Han *et al*., 2011; Piarpuzán *et al*., 2011).

1.3 Biofuel

The unpredictable supply of petroleum and increasing demand of this energy source as well as climate change concern have strengthened worldwide interest in alternatives to replace fossil-fuel from non-petroleum energy sources. The current dependence on fossil fuels as transportation fuel also has a deleterious effect on climate change, energy security and socio-economy. Fuel consumption has increased to almost double from 6,620 million tons of oil equivalent (Mtoe) in 1980 to 11,295 Mtoe in 2008. In 2008, the International Energy Agency estimated the increase to be 53% by 2030 (Malaysia Innovation Agency, 2011). As a result, the need to find alternatives to petroleum has never been more urgent.

The pursuit for a commercially viable method for the production of biofuel to replace fossil fuel as an energy source continues unabated on account of unpredictable petroleum prices and uncertain supplies, climate change and energy security concerns (Zecca and Chiari, 2010; Wuebbles and Jain, 2011; Escoba *et al*., 2009; Kim and Dale, 2004). Biofuel is a liquid fuel for the transport sector that is produced from renewable sources such as vegetable oil and biomass (Demirbas, 2007). Biofuel offers availability as it is derived from renewable sources, representing CO_2 cycle in combustion, environmental-friendly, biodegradable and is a sustainable form of energy (Puppan, 2002). The difference between petroleum and biofuel is that biofuels are from a non-polluting source and contain higher oxygen content for better combustion and reduce hydrocarbon emission compared to petroleum (Demirbas *et al*., 2009)

1.4 First Generation Biofuel

The first generation of biofuel is derived from alcohol produced from sugar that is extracted from sugarcane, fruit and palm juice, or starch grain such as potato, rice and wheat through fermentation (Pacini and Silveira, 2011). Biofuel can help to improve domestic energy security (Naik *et al*., 2010). Its large-scale production has been well-proven and demonstrated successfully in Brazil where almost 50 billion litres were produced annually (Pacini and Silveira, 2011). In 1975, Brazil launched Brazilian Alcohol Program (PROALCOOL), biofuel from sugar cane and this program aimed to reduce petroleum oil imports due to the petroleum crisis (Goldemberg *et al*., 2004). However, the food-versus-fuel program has been roundly criticized due to rising food prices leading to geopolitical instability.

1.5 Second Generation Biofuel

Malaysia produced a large amount of lignocellulosic waste mainly palm oil waste which makes up the bulk of cheap and abundant non-food material available. The lignocellulosic waste comprises mainly of cellulose, hemicelluloses and lignin, and the conversion of these materials to second-generation biofuel offers the opportunity to replace fossil fuels without competing with food (Goh *et al*., 2010). However, second generation of biofuel is still non-commercial because of the lack of technology to make this process cost and energy-efficient.

The study and research on second generation biofuel show that in order to make the cost of biofuels more comparable with standard petroleum of petrol and diesel, a more cost-effective route needs to be developed. In addition, a renewable low-carbon energy to be used for road transport is clearly needed. Second generation biofuel in the form of cellulosic ethanol could produce 75% less CO_2 than normal gasoline, whereas corn, cassava or sugarcane ethanol reduces CO_2 levels by just 60% (Patumsawad, 2011).

1.6 National Biomass Policy

The implementation of a renewable energy policy is of strategic importance for many countries as a means to diversify and wean away from the use of fossil fuels such petroleum. Greenhouse gases emission and geo-politics are contributing factors in the effort to enhance energy security (Mohammed *et al*., 2011). To enhance energy security, the US, European Union countries, ASEAN countries, Brazil and most of the countries in the world have shifted their dependency on fossil fuel to biofuel as an

alternative for energy generation. First generation biofuel's impact on land use, biodiversity, carbon balance and rising food prices have cast a negative perception on the biofuel industry.

Second generation biofuel that depends on lignocelluloses feedstock is touted as a saviour to the continuing hopes of the biofuel industry (Naik *et al*., 2010). The drive for research and development of second generation biofuel is dictated by lower cost and greater abundance of material resources for biofuel production. As the technology for second generation biofuel is yet to mature, research on biofuel production from lignocelluloses biomass continues to show positive growth throughout the world.

In Malaysia, petroleum is highly subsidized where subsidies in 2006 totalled US$4.3 billion and in 2007 totalled US$4.7 billion. The price for petroleum set by the government is far lower compared to the international price. The integration of biofuel initiative was meant to lower the amount spent on petroleum subsidies (Hashim and Ho, 2011). Countries with abundant lignocelluloses biomass resources such as Malaysia, have taken the lead in developing national biomass strategies. The Malaysian government has been experimenting with biofuel production since 1982 (Craven, 2011). Oil palm cultivation in Malaysia provides an abundant and renewable supply of waste lignocellulosic biomass, a key bioresource that has been identified for exploitation under the National Biomass Strategy's wealth creation scheme (Malaysia Innovation Agency, 2011; Sorda *et al*., 2010).

The demand for biofuel in the European market was estimated to jump from 3 million tonnes in 2005 to 10 million tonnes by 2010 (Craven, 2011). Biomass Power

Generation & Cogeneration Project (Biogenic) was jointly funded by the Government of Malaysia, United Nations Development Program, Global Environment Facility and the Malaysian private sectors to reduce the GHG emission from fossil fuel combustion processes and reduce waste residues from palm oil (PTM, 2004). Currently, the project on new wealth creation from the Malaysian's palm oil industry predicts that biofuel production from lignocelluloses biomass will be commercially viable between 2013 and 2015.

Despite uncertainties and setbacks on the development of the technology to produce the lignocellulosic biofuel, the Malaysian government continues to be optimistic towards the implementation of the second generation biofuel for commercialization. Bio-based chemicals derived from lignocelluloses biomass are also poised for commercialization between 2015 and 2020 with the objectives to increase total biomass production from 80 million tonnes in 2015 to 100 million tonnes with the proportion of products being 18% bio based chemicals, 12% bio fuel, 32% pelletization, 10% wood product and the other 28% bioenergy. This programme will add RM30 billion more to Malaysia's Gross National Income (GNI) in the year 2020 and create 66000 of job opportunities (Malaysia Innovation Agency, 2011).

1.7 Research Objective

This research is in tandem with current efforts in several parts of the world which focus on discovering new chemical methods for hydrolysing cellulose to sugars in order to ensure a sustainable supply of transportation fuel and avert climate change. The objective of this research is to optimise the parameters of perchloric acid hydrolysis of

cellulosic biomass derived from oil palm empty fruit bunch in order to maximize the yield of glucose. The glucose produced will then be subjected to fermentation for ethanol production.

1.8 Research Scope

i) To investigate the effect of perchloric acid to hydrolyse OPEFB biomass by varying the following parameters:
   - Acid concentration
   - Reaction time

ii) To compare the effectiveness of different acids for hydrolysis of OPEFB.

iii) To compare yield of ethanol after detoxification.

iv) To optimise the combination process of acid hydrolysis parameters in order to obtain highest yield of glucose.

1.9 Organisation of the Study

This thesis is organised into five chapters. Chapter one provides background of the study and discusses the generation of bioethanol and strategy of governments to overcome the uncertainties of petroleum supply.

Chapter two reviews the literature on previous study which deals with treatment, hydrolysis and fermentation of lignocellulosic biomass. It involves details in content on lignocellulosic biomass and as well as researchers involved in this area.

Chapter three describes the materials and methodology involved in conversion of OPEFB to sugars and ethanol. The analytical method includes comparisons between acids, parameters to hydrolyze OPEFB, detoxification and fermentation process.

Chapter four presents results and discussion. The results on hydrolysis between comparative acids and condition parameters of acid are reported. The effectiveness of hydrolysis is compared and the highest yield of glucose is identified. The optimum values for the variables are obtained respectively.

Chapter five concludes the study. It presents the conclusion of the objective and recommendation for further improvement of this work in the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Lignocellulose

Lignocelluloses material in plant fiber mainly consists of cellulose and hemicellulose of sugar polymer; and usually accounts for 65-70 percent of the plant dry weight. These polymer sugars mainly consist of D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, D-glucuronic acid, L-rhamnose and D-fucose. Table 2.1 shows the sugar content of different plant holocelluloses (Park and Kim, 2012).

Table 2.1: Sugar content of different plant holocellulose (Park and Kim, 2012)

	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

Lignocellulose can be liberated by hydrolysis for fermentation by microorganisms to form different chemicals. Cellulose, hemicellulose, and lignin in plant biomass can be converted to energy resources such as alcohol, methane, furfural and organic acids (Park and Kim 2012).

2.2 Cellulose

Cellulose is a straight chain polymer of D-glucose unit with linkage through several hundred to over ten thousand of β (1→4)-glycosidic bonds (Balat *et al*., 2008, Rowell *et al*., 2005). It has hydrophilic properties, insoluble in water and most organic solvents, with the contact angle of 20–30 chiral and is biodegradable. These glucose units can be broken down chemically by high concentrated acids or low concentrated acid at high temperature. Compared with starch, starch consists of D-glucose unit link with α (1→4)-glycosidic bonds, and no coiling or branching occurs.

In cellulose, the bond was 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 as shown in Figure 2.1. This bond makes cellulose results in a high tensile strength, crystalline, strong and difficult to depolymerize (Ga´mez, 2006; Patumsawad, 2011). Cellulose chain length depends on total glucose units that were linked to form polymer such as wood pulp which consist of 300 to 1700 units, of cotton and other plant fibers and a have chain length from 800 to 10,000 units.

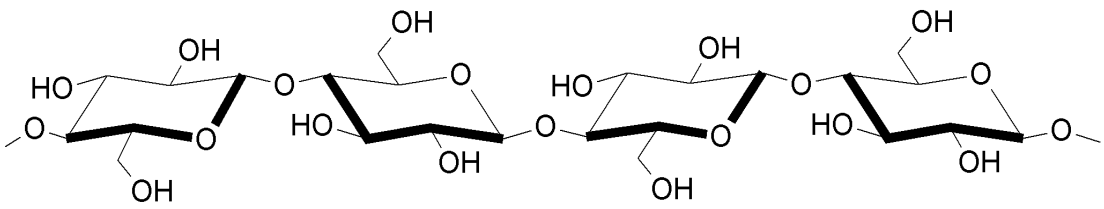


Figure 2.1: The chemical structure of cellulose.

2.3 Hemicellulose

Hemicelluloses are heteropolymers with matrix polysaccharides in almost all plant cell walls. Hemicellulose has a random structure of branched sugars polymer such as xylose and arabinose. These amorphous polysaccharides make hemicelluloses hydrolyzed easily by dilute acid, base and hemicellulose enzymes. Compared to cellulose which only contains glucose unit, hemicelluloses contains many different pentose sugar monomer which includes xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan as shown in Figure 2.2. In hemicellulose, xylose is a major D-pentose sugar monomer followed by L-sugars of arabinose. In addition, hemicelluloses also contains small amount of mannuronic acid and galacturonic acid (Gírio *et al*.,2010).

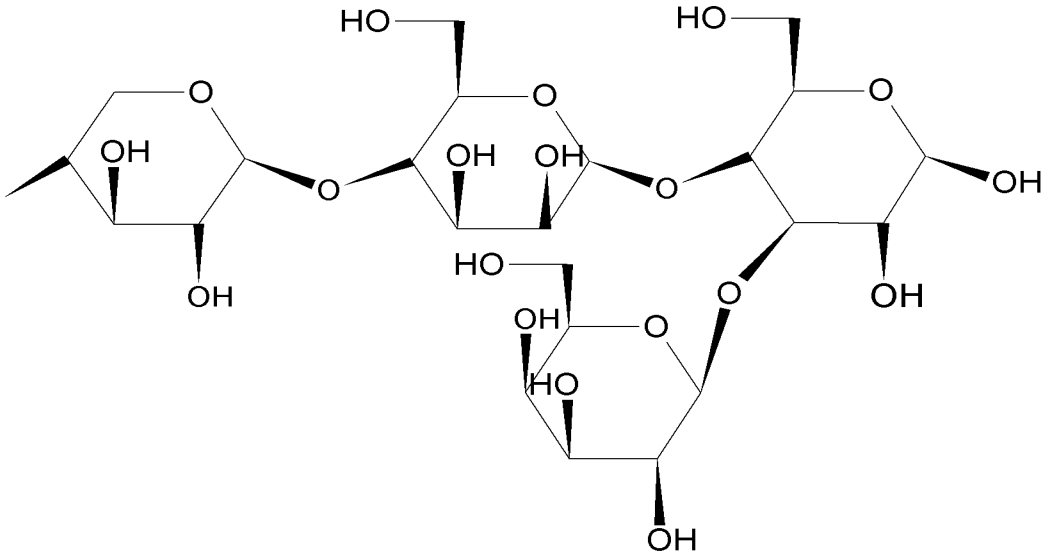


Figure 2.2: Chemical structure of hemicellulose.

2.4 Lignin

Lignin is an amorphous highly complex aromatic structure polymer connected mainly by three-dimensional cross-linked C-C and C-O-C of p-hydroxyphenylpropanaid units. It is hydrophobic and can be classified by its variable structural element. There are three basic building blocks of precursor alcohol of guaiacyl, syringyl, and p –hydroxyphenyl moieties in plant lignin. The guaiacyl units is consist of 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol; syringly unit is 3, 5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol, and p-hydroxyphenyl unit of p-hydroxycinnamyl (coumaryl) as shown in Figure 2.3 (Lewis and Yamamoto, 1990, Rencoret *et al*., 2011; Boerjan *et al*., 2003; Ralph *et al*., 2004). Lignins are soluble in base solution which it makes it easy for to be removed from lignocellulose plant biomass.

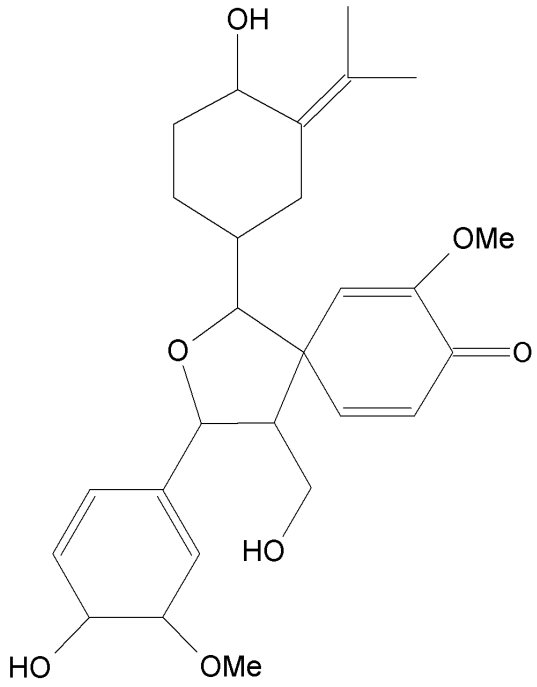


Figure 2.3: Chemical structure of lignin.

2.5 Process Conversion of Lignocellulosic Biomass

Biomass consists of complex carbohydrates polymers cellulose, hemicellulose and lignin. In lignocelluloses biomass, lignin has a reputation of hindering OPEFB saccharafication into monomeric sugars. The main function of pre-treatment is to break the ether bonds that cross-link lignin and hemicellulose, decrease crystalline of cellulose and increase biomass surface area (Mosier *et al*., 2005). Enzymes cannot efficiently convert the lignocelluloses biomass due to complex structure of lignocellulose in plant.

A variety of pre-treatment methods are applied to lignocelluloses feedstocks in order to enhance the sugar yield for ethanol production. The production of bioethanol from cellulosic biomass generally follows well-established practices: grinding and milling of crop residues to powder, chemical pre-treatment, chemical and/or enzymatic hydrolysis followed by fermentation (Chandel *et al*., 2007). The treatments include thermal treatment and chemical treatment; such as lime treatment, acid treatment, ammonia fiber explosion (AFEX) and aqueous ammonia recycle (ARP). Each pre-treatment method may have different effect on glucose and xylose yield upon enzymatic and chemical hydrolysis depending on lignocellulosic structure in plant biomass and formation of inhibitory compounds. Pre-treatment or delignification releases lignin from polysaccharides which involves the cleavage of non-phenolic β-O-linkage and phenolic α-O-4 linkage.

2.5.1 Physical Treatment

The biomass is required to undergo 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 solid biomass particle (Najafpour *et al*., 2007). Practically, physical pre-treatment was conducted by mechanical process such as chipping, grinding and explosion. A combination of chipping, grinding and milling in biomass reduces cellulose crystalline for further process. Usually, size of the biomass materials is 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Compared to ordinary ball milling, vibratory ball milling is more effective for improving digestibility by breaking down the cellulose crystalline of biomass such as spruce and aspen chips (Abasaeed *et al*., 1991). However, it depends on size and waste biomass characteristics.

2.5.2 Hydrothermal

Hydrothermal is the process of using liquid hot water (auto hydrolysis) and steam explosion treatment. The process of treatment involves compressing hot air into material at a temperature of between 150-230˚C (Garrote *et al*., 1999). The reaction takes place when hydronium ions generate in-situ by water auto ionization.

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 takes place for several seconds to a few minutes at a temperature of 160–260˚C with pressure 0.69–4.83 MPa. After several seconds to

few minutes, pressure is released to the atmospheric pressure and biomass structure explodes. 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 be achieved by either high temperature of 270˚C for 1 min or lower temperature of 190˚C for 10 min (Gírio *et al*., 2010; Dale and Moelhman, 2010; Jin and Chen, 2006).

2.5.3 Alkaline treatment

The alkaline treatment can be done by using alkaline earth metals based agents and ammonia. Most lime treatment used sodium, calcium and potassium. Alkaline treatment readily removes lignin and increases efficiency of enzyme saccharafication compared to acid treatment (Sun and Cheng, 2002). However, without proper delignification, there will be loss of some of the sugars and the production of bio ethanol is lower (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 and Holtzapple , 2010; Saha and Cotta,2008).

Lime will saponify the uronic ester linkages in 4-O-methyl-D-glucoronic acids cross along xylan hemicellulose and other polymeric materials (Misson *et al*., 2009). For example, the alkali treatment with 10 M sodium hydroxide at room temperature efficiently removes lignin from the dilute sulphuric acid-treated OPEFB fiber and decreases lignin concentration. Sodium hydroxide treatment also efficiently removes lignin from OPEPFB fiber where 85.2 g cellulose and 1.8 g hemicellulose per 100 g is

obtained in treated OPEFB fiber (Kim and Kim, 2012). After lime pre-treatment, solid separation step and pH reduction is necessary to reduce the cost of the process (Park and Kim, 2012).

Among the chemical pre-treatment methods, alkaline treatment has been proven to effectively remove lignin for conversion by biological lignocelluloses to sugar. It was promising improvement of anaerobic digestion of newspaper, corn stalk, hardwoods, softwood, and paper tubes (Fox *et al*., 2003; Qingming, 2005; Teghammar *et al*., 2009; Mirahmadi *et al*, 2010). Pre-treatment can be successfully used for high concentration or low concentration of NaOH (Mirahmadi *et al*., 2010). Typically, low concentration of NaOH, ranging between 0.5-4%, needs a high temperature and pressure to disintegrate lignin and hemicellulose for destruction of lignocellulose (Taherzadeh and Karimi, 2008).

However, in this process, no NaOH is reusable and several different inhibitors are formed during the process. In contrast, pre-treatment at high-concentration of NaOH pre-treatment, ranging between 6–20% only requires at ambient pressure and relatively low temperature. This process is efficient for the reduction of cellulose crysatillinity (Mirahmadi *et al*., 2010). In addition, high concentration NaOH pre-treatment is economical due to the possibility of reusing the NaOH solution for environmental impact in the process.

2.5.4 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*., 2011a; Li and Kim, 2011). The research on optimization pre-treatment condition of aqueous ammonia for OPEFB on temperature, time, solid to liquid ratio and ammonia concentration by enzymatic digestibility test were studied. Aqueous ammonia with concentration of 21% at 60˚C for 12 h can recover about 78.3% of glucan and remove 41.1% of lignin, respectively (Jung *et al*., 2011b). However, this reagent has hazardous, malodorous and corrosive properties for equipment.

In contrast to ammonia treatment, the concept of AFEX is similar to steam explosion where process need pressure to break lignocellulose structure. Typically, AFEX process was conducted at 90˚C in residence time for 30 min. It was 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 pre-treatment compared to other pre-treatment processes (Holtzapple *et al*., 1990).

After pre-treatment, aqueous ammonia can be recycled to reduce the cost and protect the environment. In an ammonia recovery process, a temperature up to 200˚C

was used to heat residual ammonia to vaporize and then it was withdrawn from the system by a pressure controller.

2.5.5 Acid treatment

The common acid treatments have been successfully developed for pre-treatment of lignocelluloses materials by using sulphuric acid in the range of 0.5-1.0%wt at 50˚C. The dilute sulphuric acid is used efficiently to improve cellulose hydrolysis to sugars and achieve high reaction rates (Esteghlalian *et al*., 1997). Temperature and acid concentration play an important role for pre-treatment process. Without proper pre-treatment, direct saccharification can suffer from low yields because of sugar decomposition. 4% (v/v) sulphuric acid treatment extracted almost 50% of the biomass as a soluble fraction.

Besides that, acid pre-treatment of hydrolyzing hemicelluloses to form xylan and less formation of inhibitor such as furfural is carried out. Less than 1 g/L furfural and 2.6 g/L acetic acid is regenerated in acid extract solution and other compounds are not detected in the solution depending on process temperature. Formation of high xylan in lignocellulosic materials as a third of the total carbohydrate to xylose is necessary to achieve favourable overall process. In order to remove some toxic compounds from the surface of dilute acid pre-treated OPEFB fiber, is soaked in water for 1 hour as this weakens and softens the fiber structure for hydrolysis process (Kim and Kim, 2012).

2.5.6 Hydrolysis

Hydrolysis is a decomposition process in which a chemical bond of compound is broken down by a reaction with water. The hydrolysis of cellulose can be defined as a cellulolysic process while the hydrolysis of cellulose or starch into glucose (sugar) may be defined as a saccharification process. The hydrolysis of cellulose to glucose only occurs at economically viable yields when a catalyst is used.

Due to the robust lignocellulosic architecture, the biomass cannot be hydrolyzed easily except under strong conditions (Yunus *et al*., 2010). Concentrated and dilute acids under appropriate conditions are able to penetrate the lignin-polysaccharide much better and break down cellulose and hemicellulose polymer into monomeric sugars without a pre-treatment process. Lignin has the reputation of hindering OPEFB saccharafication into monomeric sugars (Meunier-Goddik and Penner, 1999). The acid hydrolysis is shown to be more effective in the hydrolysis of crystalline structure of cellulose compared to enzymatic hydrolysis. The reason is because enzyme hydrolysis requires high porosity, specific surface area and a higher degree of hydration due to the stearic hindrance caused by lignin-polysaccharides linkage that limits access in particular fibrocystic enzymes to specific carbohydrates moieties leading to lower yields.

In addition, to improving the enzymatic hydrolytic efficiency, the lignin-hemicellulose network has to be loosened for the better amenability of cellulase to residual carbohydrate fraction for sugar recovery. Pre-treatment steps are required to remove lignin to enhance the enzymatic susceptibility of cellulose. Various mineral

acids such as dilute and concentrated (Rahman *et al*., 2007; Chin *et al*., 2010), hydrochloric acid (Herera *et al.,* 2004), phosphoric acid (Lenihan *et al*., 2010) and nitric acid (Hamilton *et al*., 2004; Rodriguez–Chong *et al*., 2004), have been used for hydrolysis of polysaccharides, performing at varying degree of efficiency. A previous study using phosphoric acid and sulphuric acid for hydrolysis of lignocellulosic and the neutralization of acids points to the advantage of introducing essential elements such as sulphur and phosphorus that is beneficial for microbial growth during the fermentation process (Zhang *et al*., 2012). However, strong acid hydrolysis can cause glucose to degrade rapidly and produce less desirable compound such as furfural from dehydration of pentoses and hydroxymethylfurfural from dehydration of hexoses during hydrolysis.

2.5.7 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 less corrosion of equipment (Gírio *et al*., 2010).

Usually, 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.

2.5.8 Concentrated Acid Hydrolysis

Unlike dilute 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 (Gírio *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. Up to 90 % of cellulose and hemicelluloses are degraded to their sub units after the treatment process. The low temperatures and pressure of the concentrated acid hydrolysis leads to minimised sugar degradation (Chandel *et al*., 2007). Sugars derived from this hydrolysis process are easily fermented by microorganism.

2.5.9 Sulphuric Acid

The most commonly used method to hydrolyze the lignocelluloses materials is the sulphuric acid hydrolysis method. Higher concentration of sulphuric acid is usually used in the first stage and is followed by a dilution with water in the second stage to

hydrolyze and dissolve the substrate. 60 % to 75 % concentrated sulphuric acid is used mostly for the first stage of sulphuric acid hydrolysis while 10 % and 30 % of concentrated sulphuric acid at temperature of 80^{o}C is used in the second stage of the hydrolysis to treat the substrates. Estimation of glucose yield depends on the acid concentration and also the reaction period. Based on the study of sulphuric acid hydrolysis of wood chip, about 80% of sulphuric acid is used to hydrolyze the small grinded mixed wooden chip before it is diluted with distilled water to obtain 26% (w/w) acid. After hydrolization process, filteration is needed to separate insoluble solid from sugar substrate. The pH of filtrate is highly acidic and needs to be neutralizing to around pH 6 to 8 for further fermentation process (Chin *et al*., 2010).

Based on Rahman *et al*., (2006), the hydrolysis of OPEFB biomass is produced by using 2% diluted sulfuric acid at 120˚C produced 31.1 g/L of xylose from hemicellulose and 4.0 g/L of glucose from cellulose. Estimation of sugars yield depends on the acid concentration and also the reaction period. Figure 2.4 shows the trend of acid concentration over period of time. As acid concentration increases, xylose concentration release tends to peak much faster but decreases easily if prolonged over a period of time.

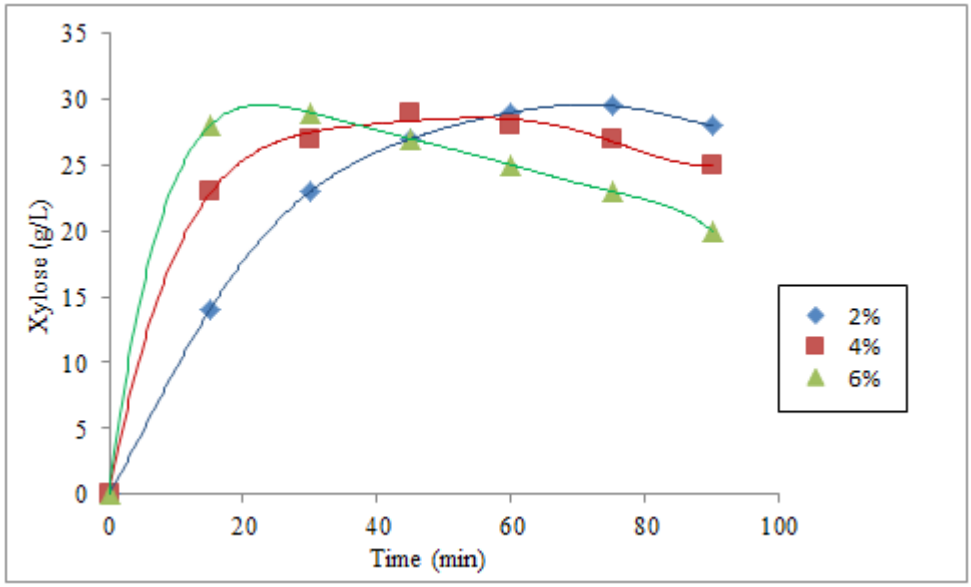


Figure 2.4: Effects of sulfuric acid concentration and reaction time on yield of xylose of OPEFB fiber at 120°C (Rahman *et al*., 2006).

In contrast, concentration of glucose increases over time and hydrolysis due to the structure of cellulose which is more crystalline and complex compared to hemicellulose. It needs a longer period of time to dilute acid to degrade cellulose to glucose as shown in Figure 2.5

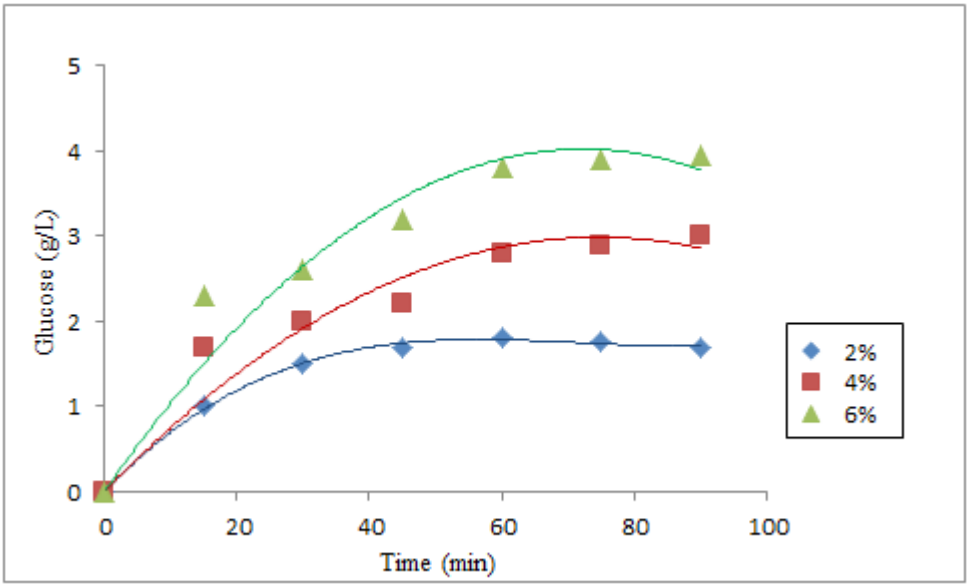


Figure 2.5: Effects of sulphuric acid concentration and reaction time on yield of glucose of OPEFB fiber at 120˚C (Rahman *et al.,* 2006).

2.5.10 Hydrochloric Acid Hydrolysis

Depending on biomass characterisation, hydrochloric acid (HCl) hydrolysis can be done at low concentration or even at high concentration of HCl. It was reported that low concentration of HCl gave low yield of sugars which only accounts for 2.7 g/L in hydrolysis of OPEFB. While high concentration of HCl can generate high sugar concentration which can account for about 20 g/L. In spite of this, high acid concentration can decrease the sugar concentration when it reacts at elevated temperatures. When the reaction takes place for a much longer time when it is associated with high temperature it is known that deformation of sugar to unwanted products such as furfural and hydroxyl methyl furfural (HMF) can take place which subsequently reduces the sugar concentration. Hydroxyl methyl furfural and furfural originate from xylose and glucose decomposition and is usually submerged or produced

when hydrolysis is conducted with the presence of acid catalyst and at high temperatures (Najafpour *et al*., 2007)

Moderate reaction with ambient pressure and temperature is preferred when concentrated hydrochloric acid hydrolysis is conducted. OPEFB lignocellulose fiber conversion of 36, 60, 65 and 80 % is achieved when 5 % solid is used with 15, 20, 25 and 30 % of concentrated HCl at a reaction time of 40 minutes. However, when 10% solid was used for a reaction time of over 60 min, the sugar concentration from the acid hydrolysis is known to account for a value of 22.5 g/L (Najafpour *et al*., 2007).

Based on Herrera *et al*., 2003, the effect of various concentration of HCl acid at atmospheric pressure on the hydrolysis of sorghum straw was studied in order to look on the production of glucose, xylose, furfural and also acetic acid. From the studies, when the hydrolysis is performed with 6% HCl at 100°C, xylose concentration peaks at 180 min of reaction time with a value of 19.7 g/L and then decreases over the time period such as shown on Figure 2.6. While glucose, furfural and acetic acid are released concentration is marked up to 5.3, 1.7 and 3.6 g/L at 180 min, respectively.

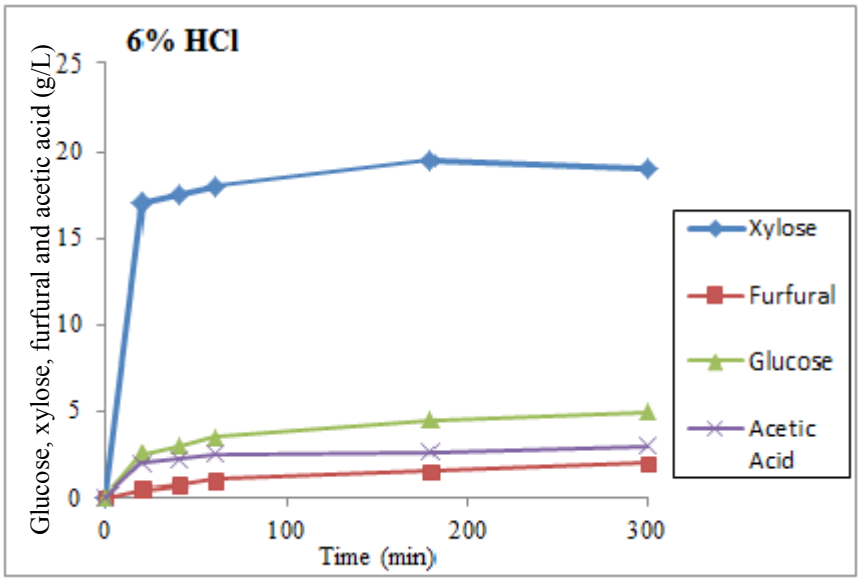


Figure 2.6: Yield of glucose, xylose, furfural and acetic acid at 6 % HCl against time of reaction at 100˚C (Herrera *et al*., 2003).

2.5.11 Perchloric Acid

Perchloric acid ($HClO_4$) usage has the potential for hydrolysis process of lignocelluloses material and this is preferable since the perchloric acid has a function as a hydrolysing agent and also as an oxidising agent. The oxidizing function of $HClO_4$ can help in delignification and can reduce the reaction time and energy if compared to the other acid pre-treatment that are used. In addition, neutralisation of the access $HClO_4$ with KOH can lead to precipitation of the insoluble $KClO_4$, and this has an advantage as it can be recycled to $HClO_4$.

Theoretically, perchloric acid hydrolysis can be conducted with high concentrations and followed by diluted perchloric acid. Based on Ismail *et al.,* (2012), hydrolysis process of wheat straw with perchloric acid was preferred to be carried out in two stages because higher sugar degradation can be avoided from the hydrolyzed materials when conducted in two stages rather than single stage. On top of that, when two-stages of hydrolysis is conducted, formation of fermentation inhibitors can be lessened and reduce the heating time (Ismail *et al*., 2012).

2.5.12 Fermentation

Fermentation is a process where the breakdown of carbohydrate such as sugar takes place with organic compounds; bacteria, yeast or other microorganism; into acid

or alcohol through oxidation reaction. In this scope of research, ethanol is the main objective of research as it is essential for bio fuel as a renewable energy for future power generation. Fermentation process is dependent on time and also conditions of the fermentation to give a high yield of fermentation product (Chin *et al*., 2011).

The pH and temperature has a significant influence on fermentation due to its effect on yeast growth, fermentation rate and by-product formation (Pramanik., 2003). From previous research conducted on the effect of temperature on the fermentation process, it is known that the temperature has proven to yield a complex mixture of products. The most efficient ethanol fermentation conditions are determined by high yield of ethanol in the shortest fermentation time.

Saccharomyces cerevisiae is a microorganism that is known to give significant effect on the fermentation process. The microorganisms has attracted considerable attention in recent years for the simultaneous saccharification and fermentation (SSF) process of bio-ethanol from agricultural wastes since it is known to have a higher tolerance to both ethanol and also inhibitors that is present in hydrolysates of the lignocellulosic materials. Based on Millati *et al*., (2011), the medium has been pH adjusted and sterilized to ensure that the fermentation process yields a high efficiency. Other known fungus used for fermentation process is *Mucor indicus*.

From the research, it was reported that both *S. cerevisiae* and *M. indicus* are proven to ferment the hydrolyzates from the corresponding stages despite of the presence of HMF and furfural. The yields of ethanol from the fermentation process are

0.46 g ethanol/glucose by *S. cerevisiae* and 0.45 g ethanol^{-1} xylose by *M. indicus* (Millati *et al*., 2011).

CHAPTER 3

METHODOLOGY

3.1 Materials

Oil palm empty fruit bunch is most abundant biomass waste in Malaysia. It mainly consist of cellulose and hemicellulose of sugar polymer; D-glucose, D-xylose and L-arabinose. Perchloric acid has the potential to hydrolysis lignocellulose material to monomer sugar for bioethanol production through fermentation process. Therefore, OPEFB was used as raw material to study efficiency of perchloric, sulphuric and hydrochloric acid toward lignocellulosic material.

Oil palm empty fruit bunches (OPEFBs) were provided by Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. The OPEFBs were then sun-dried, grounded by knife mill, and sieved to obtain a particle size of approximately 1.0 mm. The grounded OPEFBs were then dried in an oven overnight at 80˚C.

OPEFB was ground to particle size of approximately 1.0 mm and dried in an oven at 80˚C for overnight.

↓

For first stage,

1 g of OPEFB hydrolysed with 10 mL of 10 M perchloric acid and placed in water bath (30˚C) for 30 min.

For second stage,

The solution was then diluted with distilled water to give concentration of 5%, 7%, 9% and 12% (v/v) and heated to 100˚C for 90, 180, 270 and 300 min intervals.

The hydrolysate was neutralized with 1M KOH and filtered.

Analysis using HPLC for sugars determination.

Detoxification by using activated charcoal

Fermentation by *Saccharomyces cerevisae* strain S4.

The fermented solution was analyzed with HPLC for ethanol quantitation.

Figure 3.1: Overall methodology layout for study on perchloric acid

OPEFB was ground to particle size of approximately 1.0 mm and dried in an oven at 80˚C for overnight.

For first stage,

1 g of OPEFB hydrolysed with 10 mL of 10 M sulphuric or hydrochloric acid and placed in water bath (30˚C) for 30 min.

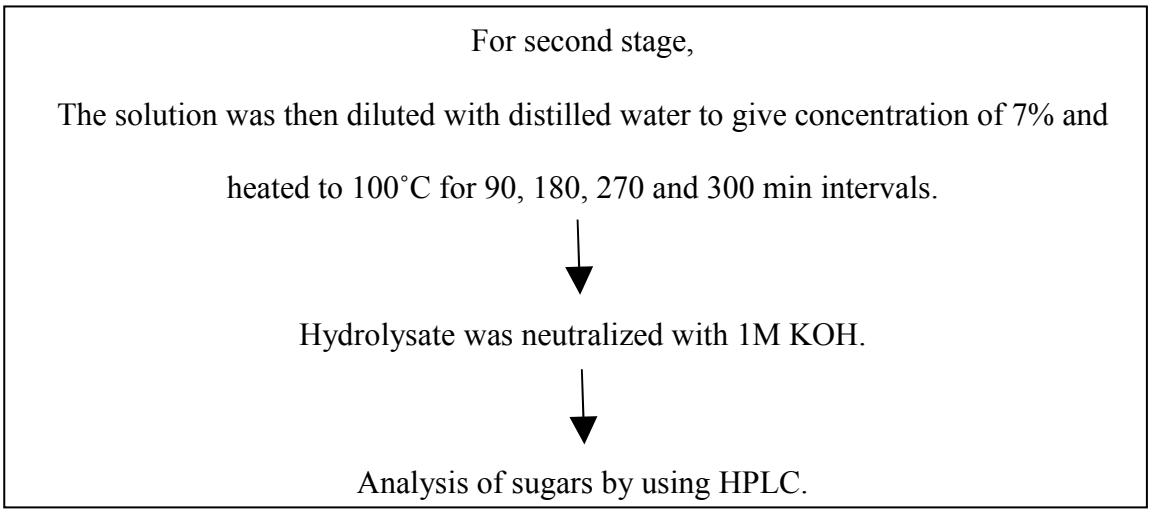


Figure 3.2: Overall methodology layout for study on comparison between acid.

3.2 Chemicals and Reagents

Standard sugars such as glucose, xylose and arabinose were purchased from Sigma-Aldrich Chemical Inc., US). Chemicals such as perchloric acid, hydrochloric acid, sulphuric acid and potassium hydroxide were purchased from Merck, Germany.

3.3 OPEFB Hydrolysis with Perchloric Acid by Variation in Acid Concentration and Time of Reaction

The method of hydrolysis using perchloric acid was adapted from patent PCT/MY2010/000018 and amendments were made in concentration of acid and procedures in the hydrolysis of biomass (Arifin and Teoh, 2009). For the first stage hydrolysis, 1 g of OPEFB samples were weighed, placed in test tubes and treated with 10 mL of 10 M perchloric acid (Najafpour *et al*., 2007). The sample was then placed in a water bath at 30˚C for 30 min and stirred for every 10 min.

For second stage hydrolysis, distilled water was then added to dilute the solutions to give acid concentrations of 5%, 7%, 9% and 12% (v/v). The sample tubes were then fitted with rubber stoppers and heated to 100˚C for 90, 180, 270 and 300 min intervals. Each test was repeated thrice.

3.4 Neutralization of the Hydrolysate

The acidic mixtures from the above reactions were neutralized with ice-chilled 100 mL of 1M KOH. Continuous stirring and cooling were maintained during the neutralization process. Upon neutralization, potassium perchlorate precipitated and was filtered off. The filtrate obtained was dark gold-coloured sugary solution. The precipitate residues washed with distilled water to recover more sugars and combined with the mother filtrate as total sugars of 250 mL.

3.5 Analytical Method

The qualitative analysis of products after the hydrolysis of OPEFB were analysed by High Pressure Liquid Chromatography (HPLC), Waters with Refractive Index, RI as the detector while the quantitative analysis for sugars were determined. The products obtained after fermentation were also determined by HPLC qualitatively and quantitatively. The amount of monosaccharide and ethanol were analyzed using the REZEX ROA-Organic Acid H+ (8%) 0.45 µm, 150 mm × 7.8 mm column and the products were determined by a refractive index detector at 30˚C. The mobile phase used was degassed 0.005 N sulphuric acid at a flow rate of 0.8 ml/min and oven temperature was maintained at 80˚C. All sample and standard were filtered through 0.45 µm nylon

membrane filter prior to analysis and 20 µL of the sample was injected. The sugars produced were identified using the retention time of standard chemicals for D-xylose, L-arabinose and D-glucose. The column was specialized for sugar analysis and retention time of standard sugars can be used to identify sugars in hydrolysate.

The quantitative measurement of sugars, ethanol and by-products were calculated using a calibration curve of each individual standards according to the equation,

$$\frac{I_{HPLC\ (\text{sample})}}{I_{HPLC\ (\text{ref})}} \times \frac{c_{\text{ref}}}{1000} \times \frac{V_{\text{total}}}{m_{\text{dry biomass}}} \times f_{\text{dilution}} \times 100\,\%$$

where $I_{\text{HPLC (sample)}}$ is the intensity of sugars or ethanol samples in mV.s; $I_{\text{HPLC (ref)}}$ is the intensity of sugars or ethanol references in mV.s; c_{ref} is the concentration of sugars or ethanol references in mg mL^{-1}; V_{total} is the total volume of sugars or ethanol samples in mL; $m_{\text{dry biomass}}$ is the mass of dry biomass in g; and f_{dilution} is the dilution factor. The analyses were performed for all the samples in triplicate.

3.6 Comparison by Using Sulphuric and Hydrochloric Acid

For first stage hydrolysis, 1 g of OPEFB were weighed, placed in test tubes and added with 10 mL of 10 M sulphuric acid. The sample was then placed in a water bath at 30˚C for 30 min and stirred for every 10 min.

For second stage hydrolysis, distilled water was then added to dilute the solutions to give acid concentrations of 9% (v/v). The sample tubes were then fitted with rubber stoppers and heated to 100˚C for 90, 180, 270 and 300 min intervals. The solutions were

neutralized with ice-chilled approximately 100 mL of 1M KOH and each test was repeated thrice. This method was repeated using hydrochloric acid.

3.7 Detoxification using activated carbon

After neutralization, the hydrolysates were detoxified with 3.5% (w/v) activated charcoal and stirred for a period of 1 hour. The hydrolysates were filtered using a vacuum filtration system (Phenomenex). The total amount of inhibitor before and after detoxification were estimated from the fermentation studies using HPLC.

3.8 Fermentation of Cellulose Hydrolysates

Saccharomyces cerevisae strain S41 was inoculated in a 2 L conical flask containing 1L Yeast Mold (YM) broth and incubated in a 32 ºC incubator shaker for 48 hours. The cells were harvested by centrifugation at 10,000 rpm using 250 mL centrifuge bottles. 1% cells (w/v) were inoculated into 100 mL conical flasks containing the hydrolysates (Watanabe *et al.,* 2007).

The percentage of ethanol yield of glucose was calculated using Eq. (1) (Park *et al*., 2010) :

$$\text{Ethanol Yield} = \frac{\text{ethanol of produced (g) after fermentation} \times 100}{\text{the amount of glucose in efb} \times 0.511} \qquad \text{Eq (1)}$$

where 0.511 is ratio of conversion of conversion of glucose to ethanol.

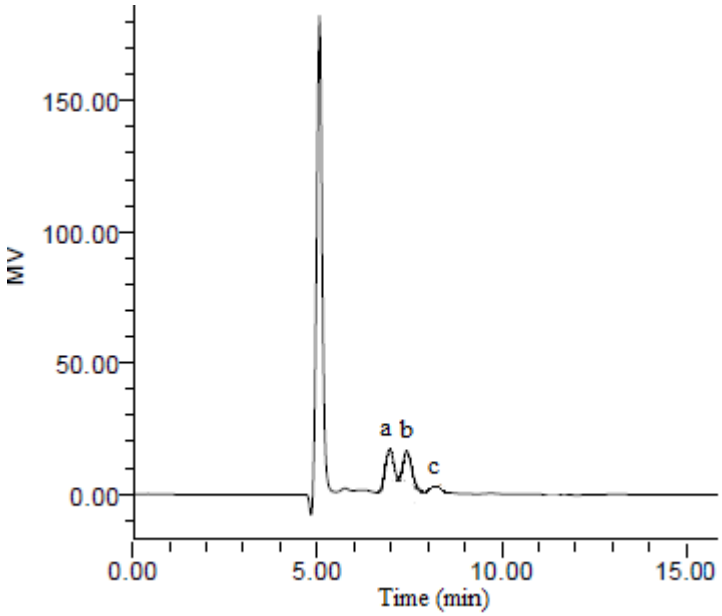
CHAPTER 4

RESULTS AND DISCUSSION

4.1 Qualitative analysis

According to Figure 4.1, the sugars can be separated by using the REZEX-ROA organic acid column and the qualitative measurement of sugars were identified by HPLC-grade sugars standards. The hydrolysate from OPEFB consist of glucose, xylose and arabinose in which the retention time of glucose, xylose and arabinose were around 6.9 mins, 7.4 mins and 8.1 mins, respectively. These were confirmed by the glucose, xylose and arabinose standard references.

Figure 4.1: Chromatogram of standard sugars a) Glucose; b) Xylose and c) Arabinose



4.2 Effect of Concentration and Time on Yield of Sugars in Perchloric Acid Hydrolysis

The perchloric acid hydrolysis of OPEFB was carried out using different acid concentrations and different time-frames. As shown in Table 4.1, there is a steep increase in sugars production for the first 30 min for all four concentrations of perchloric acid while the rate of sugar production increases less steeply from then on. For both 5 and 7 % perchloric acid concentrations, the increase in sugar yield starts to saturate from 90 min onwards. However for 9 and 12 % perchloric acid concentrations, maximum peaks are obtain for both cases at approximately 180 min. For both perchloric acid concentration, the peaks start to decrease with the increase in time except for the 12 % perchloric acid concentration whose peak decline in more pronounced manner. This decrease is attributed to the degradation of sugars over time because of the oxidative properties of perchloric acid above 9 % at a temperature 100˚C. The highest yield of sugar produced was 43.7 g per 100 g of dry OPEFB at 12 % acid concentration and 180 min.

A lesser yield of sugars of 33.2 g and 37.0 g per 100 g of dry OPEFB at 360 min were obtained for the lower acid concentration at 5 % and 7 % respectively. It was indicates that sugar concentration will be increased by increasing the acid concentration. This is because of acid was proportional to H+ and the more hydogen ions in solution lead the more rapid hydrolysis occurred and break the glycosidic bond of hemicellulose into fraction of xylose and arabinose (Najafpour *et al*., 2007). In addition, these hydrogen ions were able to cleave O-ether bonds between cellulose, hemicellulose and lignin thereby distrupting the cellulose structure to form glucose.

Table 4.1: Total yield of sugars (g) at various concentration perchloric acid and reaction time.

Acid	Time (min)

CConcentration (%)	30	90	180	270	360
5	25.192	31.456	32.129	32.146	33.542
	24.321	30.129	33.412	31.456	33.098
	26.720	30.890	32.292	33.394	32.834
Average	25.411±1.21	30.825±0.67	32.611±0.70	32.332±0.98	33.158±0.36
7	26.542	31.698	35.678	36.109	37.754
	26.754	31.260	35.109	35.109	36.567
	26.600	32.205	35.529	35.666	36.802
Average	26.632±0.11	31.721±0.47	35.4385±0.30	35.628±0.50	37.041±0.63
9	27.543	33.187	42.109	38.450	30.451
	28.752	34.987	42.987	39.109	31.126
	29.154	33.868	42.743	39.996	30.724
Average	28.483±0.84	34.014±0.91	42.613±0.45	39.185±0.63	30.767±0.34
12	31.702	36.709	43.657	33.870	29.870
	30.010	35.977	43.798	34.532	28.409
	29.254	35.919	43.564	33.568	29.846
Average	30.322±1.25	36.202±0.44	43.673±0.12	33.99±0.49	29.375±0.84

4.3 Production of Glucose, Xylose and Arabinose

The composition of oil palm empty fruit bunch is mainly composed of cellulose, hemicellulose and lignin. Cellulose derived from monomer of glucose and linked by glycosidic bond. Based on concentration and rate studies, using perchloric acid indicates that the cellulosic content in oil palm empty fruit bunch that is amenable to hydrolysis is of about 25% by weight.

Table 4.2 illustrates the yield of glucose on the effect of acid concentration and rate of reaction. The yield of glucose at 5% and 7% of acid concentration keep on increasing with time and reached 16.3 g and 19.0 g per 100 g of dry OPEFB at 360 min. Increasing the acid concentration to 9% and 12% reduces the reaction times to reach peak yields at 180 min with 20.8 g and 22.1 g per 100 g of dry OPEFB of glucose

respectively. However, the effect of acid concentration has a detrimental effect on glucose yield especially after 180 min whereby the yield of glucose decreases over time due to the oxidative degradation by perchloric acid. For the case at 5% and 7% perchloric acid concentration, the yield of glucose will increase if the reaction was extended beyond 400 min.

Table 4.2: Total yield of glucose at variation concentration perchloric acid and reaction time.

Acid Concentration (%)	Time (min)				
	30	90	180	270	360
5	7.765	9.545	12.629	14.452	16.249
	7.103	9.702	12.104	14.018	16.402
	7.527	9.787	11.411	13.977	16.381
Average	7.465±0.34	9.678±0.12	12.048±0.61	14.149±0.26	16.344±0.08
7	8.343	9.545	14.384	16.402	18.988
	7.982	9.102	14.252	16.459	18.462
	8.044	9.034	14.225	16.453	19.463
Average	8.123±0.19	9.227±0.28	14.287±0.09	16.438±0.03	18.971±0.50
9	9.882	12.812	20.884	19.454	14.018
	9.102	12.108	20.097	19.102	14.323
	10.212	12.151	21.362	18.951	14.757
Average	9.732±0.57	12.357±0.39	20.781±0.64	19.169±0.26	14.366±0.37
12	10.782	13.462	22.452	16.405	12.702
	10.078	13.676	22.107	16.884	12.108
	10.526	13.428	21.801	16.031	12.855
Average	10.462±0.36	13.522±0.13	22.12±0.33	16.44±0.43	12.555±0.39

The hemicellulose portion of OPEFB is mainly made up of monosaccharides of xylose and arabinose. Xylose is the major sugar component produced while arabinose is minor sugar produced by acid hydrolysis in our experiment. Table 4.3 shows that the initial yield of xylose was 15.4 g per 100 g of dry OPEFB for all acid concentrations. From Table 4.3, it shown after 90 min of heating, the xylose yield peaked for all acid concentrations of 5, 7, 9 and 12% yielding 20.4 g, 21.9 g, 21.0 g and 22.1 g per 100 g of dry OPEFB of xylose, respectively. After 90 min of heating, hemicellulose is fully hydrolyze to xylose. This is because the structure of hemicellulose is randomly packed and it was required shorter reaction time for hemicellulose to convert to xylose. However, a gradual decrease in xylose concentration was observed for all acid concentrations and after 360 min, the yield dropped by approximately 23% due to destruction of xylose.

Table 4.4 shows that the initial yield of arabinose is 0.7 g and peaked at 0.82 g, 0.86 g, 0.86 g and 0.87 g per 100 g of dry OPEFB for 5, 7, 9 and 12% acid concentrations respectively. The arabinose yields dropped steeply after 30 min for all acid concentrations indicating the propensity of arabinose to oxidative destruction by perchloric acid. In fact after 360 min, 75% of arabinose was destroyed under these conditions. The bonding of xylose and arabinose in hemicellulose is randomly packed and less crystalline rendering it to be more prone to degradation compared to cellulose. Figure 4.2 shows the chromatogram of hydrolysis of dry OPEFB with 12% of perchloric acid at 180 min.

Table 4.3: Total yield of xylose (g) at variation concentration perchloric acid and reaction time.

Acid Concentr-ation (%)	Time (min)				
	30	90	180	270	360
5	17.023	20.679	19.787	17.354	16.459
	17.455	20.846	20.012	17.678	16.667
	16.891	19.765	19.955	17.588	16.479
Average	17.123±0.29	20.43±0.58	19.918±0.12	17.54±0.17	16.535±0.11
7	17.776	21.945	20.545	19.012	17.923
	17.543	21.322	20.897	18.239	17.215
	17.631	22.31	20.1765	18.525	18.361
Average	17.65±0.16	21.859±0.50	20.5395±0.36	18.592±0.39	17.833±0.58
9	17.733	20.779	21.109	19.747	16.182
	17.901	21.231	21.322	19.765	16.097
	18.036	21.077	21.265	19.261	16.267
Average	17.89±0.15	21.029±0.23	21.232±0.11	19.591±0.29	16.182±0.09
12	18.525	22.109	20.945	16.918	16.473
	19.017	21.945	21.017	17.305	16.028
	19.419	22.2145	21.251	17.398	17.338
Average	18.987±0.45	22.0895±0.14	21.071±0.16	17.207±0.25	16.613±0.67

Table 4.4: Total yield of arabinose (g) at variation concentration perchloric acid and reaction time.

Acid Concentration (%)	Time (min)				
	30	90	180	270	360
5	0.815	0.702	0.65	0.645	0.288
	0.846	0.732	0.672	0.617	0.241
	0.808	0.717	0.613	0.667	0.308
Average	0.823±0.02	0.717±0.02	0.645±0.03	0.643±0.03	0.279±0.03
7	0.88	0.624	0.62	0.604	0.231
	0.878	0.619	0.612	0.597	0.242
	0.819	0.662	0.604	0.593	0.238
Average	0.859±0.03	0.635±0.02	0.612±0.01	0.598±.01	0.237±0.01
9	0.871	0.624	0.587	0.424	0.228
	0.864	0.618	0.609	0.417	0.207
	0.847	0.642	0.604	0.434	0.222
Average	0.861±0.01	0.628±0.012	0.600±0.01	0.425±0.01	0.219±0.01
12	0.884	0.598	0.473	0.352	0.212
	0.87	0.603	0.494	0.337	0.201
	0.865	0.565	0.479	0.34	0.208
Average	0.873±0.01	0.589±0.02	0.482±0.01	0.343±0.01	0.207±0.01

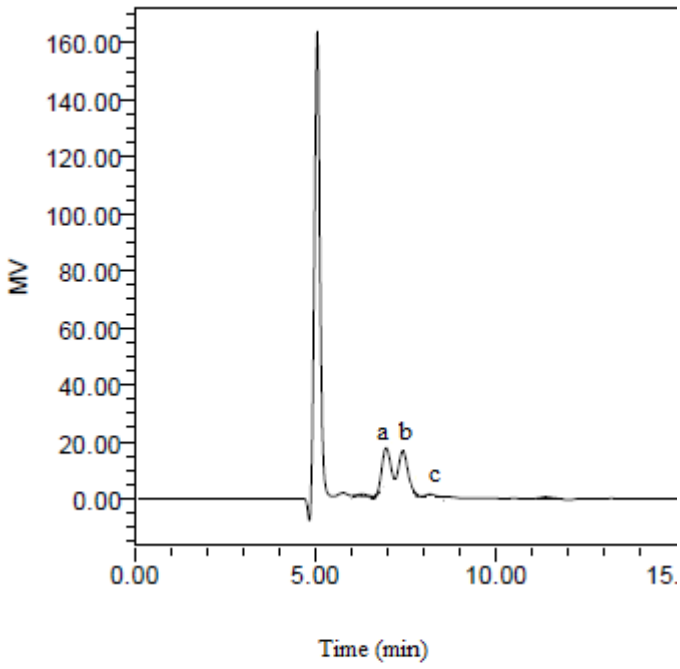


Figure 4.2: Chromatogram of sugars from hydrolysate a) Glucose; b) Xylose and c) Arabinose.

4.4 Neutralization and Fermentation

After hydrolysis, the hydrolysates were neutralised with potassium hydroxide that immediately precipitates potassium perchlorate. The mixture was filtered using over filter paper and the filtrate was added with powdered charcoal as a detoxification process before fermentation. Perchloric acid that was neutralized with potassium hydroxide to produce insoluble potassium perchlorate, a salt that is easily separated from the sugar solutions for further downstream processing.

This method is in contrast with the use of H_2SO_4 and HCl which yield soluble neutralised salts of K_2SO_4 and KCl, thus presenting separation problems from the sugars in solution. Furthermore, recycling of the salts back to the acid appears to be tedious and technically challenging. One example is the use of 77% sulphuric acid as a hydrolytic reagent as reported in the Arkenol patent. The method suffers from a

pronounced acid charring, and the neutralization of H_2SO_4 with lime solution yields an intractable precipitate of gypsum which poses separation difficulties for the sugars. The sugar targeted in this study is glucose which will be converted into ethanol. The hydrolysate with the highest yield of glucose was subjected to fermentation process. The final yield of ethanol was 11 g/L representing a conversion of 97% (based on ratio of glucose to ethanol, 0.511:1).

4.5 **Formation of Inhibitors During the Hydrolysis Process**

During acid hydrolysis, sugar degradation can cause formation of inhibitors. The formation of acetic acid and formaldehyde were found in the hydrolysates after fermentation. However, the yield of those inhibitors were lower because of the time of hydrolysis were 180 min. The inhibitor were possibility degrade and broke down into various degraded product. The degradation of inhibitor mostly converted to hydrolysate before detoxification and after detoxification. It has been seen that the yield of inhibitor before detoxification was 0.28 g/L and reduced to 0.11 g/L. A detoxification by charcoal, adsorption on active carbon, can removed phenolic compound from lignin for the growth inhibitors cited. The other inhibitors such as furfural and hydroxymethylfurfural cannot be detected by the system.

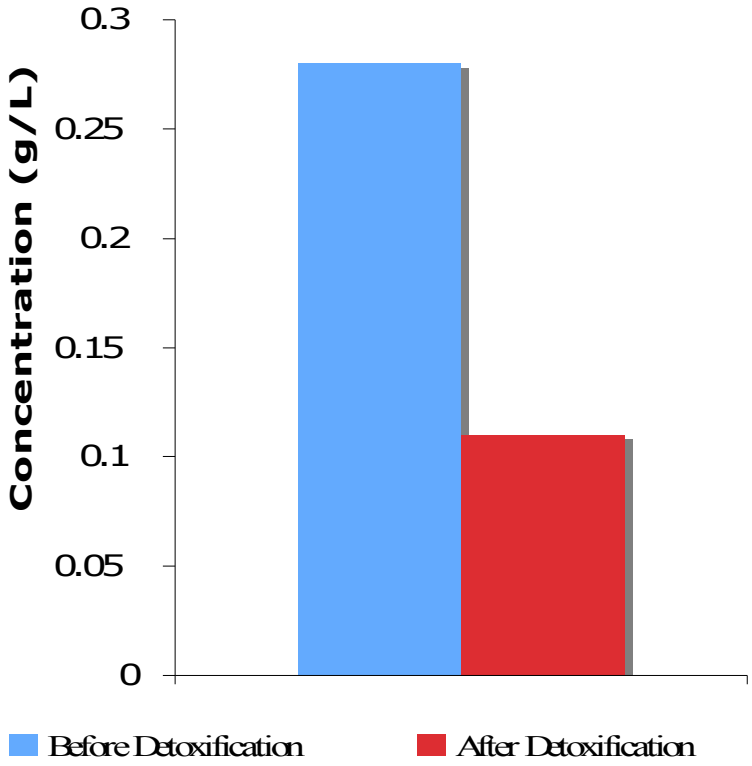


Figure 4.3: Comparison of total inhibitor before and after detoxification

4.6 Comparison Performance of Perchloric Acid, Hydrochloric Acid and Sulphuric Acid Hydrolysis of OPEFB

The 10 M perchloric acid was added to OPEFB and stirred at room temperature (30˚C) and the yield of sugars was 17.3 g per 100 g of dry OPEFB. Concentrated acid were used to break down cleave O-ether bonds between cellulose, hemicellulose and lignin due to robust lignocellulosic architecture. To prevent further destruction of sugars under 10 M perchloric acid, the mixture was diluted with distilled water to 9% perchloric acid. The resulting solution was further heated to 100˚C such as for 360 min. During this period, aliquots of solution were drawn and total sugars were determined at 30 min interval.

Initially, the yield of total sugars obtained was 17.3 g and the yield increases to 28.5 g per 100 g of dry OPEFB at 30 min as shown in Table 4.5. The peak yield is obtained at 42.6 g after 180 min, after which the yield drops to 30.8 g per 100 g of dry OPEFB after 360 min. The aqueous perchloric acid has a dual function by acting as an oxidising agent and also as a hydrolyzing agent. The drop in yield after 180 min is because of the destruction of sugars upon too long an exposure to perchloric acid. This destruction of sugars may be prevented by diluting the solution further.

Similar reaction with sulphuric acid reveals that the initial yield of total sugars is lower at 3.7 g and the yield increases to 19.5 g at 30 min, reaching the saturation level of 24.8 g per 100 g of dry OPEFB. Hydrochloric acid hydrolysis of OPEFB shows a similar pattern except the initial yield was slightly lower yield at 3.7 g of total sugars and the yield increases to 18.4 g at 30 min, reaching the saturation level of 22.3 g per 100 g of dry OPEFB. Both sulphuric and hydrochloric acid do not destroy sugars even after 360 min (27.8 g and 26.7 g per 100 g of dry OPEFB) attesting to their non-oxidative nature in contrast to perchloric acid.

The patent and other literature reveal that sulphuric acid and hydrochloric acid are commonly used in the hydrolysis of lignocellulosic biomass. Najafpour *et al*., 2007 reported that the highest yield of reducing sugar in OPEFB obtained by the hydrolysis of hydrochloric acid was 22.5 g /L, in agreement with our result. In studies conducted by others, the hydrolysis process using sulphuric acid and hydrochloric acid were conducted at high temperature and pressure. The yield of sugars will increase when pre-treatment was carried out. (Zhang *et al*., 2012).

Table 4.5: Total yield ofsugars (g) using various type of acid and reaction time.

Type of Acid	Time (min)				
	30	90	180	270	360
9% $HClO_4$	27.543	33.187	42.109	38.450	30.451
	28.752	34.987	42.987	39.109	31.126
	29.154	33.868	42.743	39.996	30.724
Average	28.483±0.84	34.014±0.91	42.613±0.45	39.185±0.63	30.767±0.34
9% H_2SO_4	20.43	24.658	25.902	26.229	27.812
	19.78	23.977	25.154	26.032	27.987
	18.23	25.579	26.437	26.066	27.628
Average	19.48±1.13	24.738±0.80	25.831±0.64	26.109±0.11	27.809±0.18
9% HCl	17.997	22.45	23.097	25.102	26.989
	18.762	22.109	23.432	24.768	27.623
	18.336	22.449	22.789	25.202	25.611
Average	18.365±0.38	22.336±0.20	23.106±0.32	25.024±0.23	26.741±1.02

CHAPTER 5

CONCLUSION

5.1 Conclusion

The digestion of crop residues to fermentable glucose monomers involves pretreatment either in the form of acid treatment or base treatment. In enzymatic methods, dilute acid or alkaline pretreatment disrupts the lignin-hemicellulosic structure and increase accessibility of the cellulose towards hydrolysis.

By using aqueous perchloric acid, pretreatment is no longer necessary as the acids act as an oxidising agent to open up the lignin-hemicelluosic structure, making way for the hydrolysis of cellulose. The aqueous perchloric acid has a dual functions, it has the ability to act both as an oxidising agent and also as a hydrolyzing agent. The judicious use of the performance parameters such as the concentration of perchloric acid and the duration of time for the hydrolysis of cellulosic biomass will fermentation process for the production of bioethanol. Perchloric acid, while expensive at about USD 800 per metric tonne compared to sulphuric and hydrochloric acid which is about USD 400 and USD 200 per metric tonne, respectively. However, it may be recycled from the neutralised form (potassium perchlorate) and can be economically recycled. This process avoids pre-treatment, a cost saving measure, and along with perchloric acid recovery, a potentially viable economical method for bioethanol production should be considered. Thus the judicious use of the concentration of perchloric acid is important to control the reaction that will lead to a minimum

concentration of inhibitors and the maximum concentration of glucose. Based on experiment, the combination of perchloric acid concentration of 12% and the time of 180 mins gave the best yield of 43.7 g/ 100 g OPEFB.

Compared with other acids such as sulphuric and hydrochloric acid, hydrolysis by using perchloric acid does not required high energy such as temperature and pressure to break down cellulose structure to glucose monomer. Sulphuric and hydrochloric acid needed high pressure or temperature to complete the reaction of hydrolysis to glucose monomer in order to get highest yield of glucose.

5.2 Future Research

Current efforts around the world focus around discovering new chemical methods and developing genetically-engineered microbes for the purpose of hydrolysing cellulose to sugars in order to ensure a sustainable supply of transportation fuel and avert climate change. Hydrolysis by using perchloric acid promises highest yield of glucose to be used for fermentation to ethanol production. The further research with large scale needed to optimise yield of sugars produced.

Biorefinery is the processes conversion of biomass to produce fuels and other value chemicals and the most important for economically in production of bioethanol. Biomass consist of lignocellulosic material such as cellulose, hemicellulose and lignin. In this study, it was focused on study of conversion of cellulose to glucose to ethanol. Lignin can be recovered by extraction in alkaline solution. Besides than cellulose, hemicellulose can be convert to other sugars such as xylose and arabinose. These sugars

can be fermented to ethanol by using specific yeast such as Phichia stipitis or Mucor indicus. The other inhibitor will be studied to increase yield of ethanol during fermentation. The solution will then be distilled until a 95% ethanol solution is obtained. After drying, the alcohol will then be blended with petrol at 5, 10 and 15% portions to give petrol blends called E5, E10 and E15.

In addition, this sugar can be converted into product of commercial value such as xylitol for odontological and pharmaceutical industries. Lignin can be extracted by using alkaline solution and converted to valuable products, such as carbon fibre and adhesive. Other by-product after fermentation such as levulinic acid, furfural, acetic acid and HMF must be studied to minimize inhibitor during fermentation process.

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APPENDIX A

PUBLICATION

Under Submission

Dayang Siti Shamsiah Awang Bujang, Teoh Teow Chong, Kamal Aziz Ketuly, Kelvin Swee Chuan Wei & Zainudin Arifin (2012). Harvesting of Biorenewable Industrial Sugars from Oil Palm Empty Fruit Bunch by Perchloric Acid Hydrolysis. *Journal Oil Palm Research* – Ref No. 7-137(E)01442.

Presentation

Oral presentation " Perchloric Acid Hydrolysis of Crop Residues for the Production of Bioethanol" - 19th IUPAC International Conference on Chemical Research Applied to World Needs 2011.