COMPARATIVE ANALYSIS OF THE BIOETHANOL YIELD AND COMPONENTS OF SUGAR EXTRACTED FROM OIL PALM FROND UNDER DIFFERENT PREHYDROLYSIS TREATMENTS

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by

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LIST OF ABBREVIATIONS

acid	Acid pretreatment
alk	Alkaline pretreatment
AP	Alkaline peroxide
ASC	Acidified sodium chlorite
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflectance
auto	Autohydrolysis pretreatment
Cho	Choline
CI	Crystallinity index
D	Delignification percentage
DAD	Diode-Array Detection
EDX	Energy Dispersive X-Ray
EFB	Empty fruit brunch
E	Ethanol yield
ELSD	Evaporative light scattering detector
Endo	Endoglucanase
Eq	Equation

Exo	Exoglucanase
FID	Flame ionization detection
FPU	Filter paper unit
FTIR	Fourier-transform infrared spectroscopy
GC	Gas chromatography
G	Glucose recovery
HMF	5-Hydroxymethylfurfural
HPLC	High-performance liquid chromatography
IL	Ionic liquid
LCB	Lignocellulosic biomass
MS	Mass spectrometry
MW	Molecular weight
NQ	Nonquantifiable
O.D	Oven dried
OAc	Acetate
OPEFB	Oil palm empty fruit bunch
OPF	Oil palm frond
OPT	Oil palm trunk
Peroxy	Alkaline peroxide pretreatment

PMP	1-Phenyl-3-Methyl-5-Pyrazolone
POME	Palm oil mill effluent
RI	Refractive index
SEM	Scanning electron microscope
SHS	Superheated steam
TAPPI	Technical Association of the Pulp and Paper Industry
UV	Ultraviolet
Vis	Visible
XRD	X-ray diffraction
β-glu	Beta-glucosidase

LIST OF SYMBOLS

A	Ampere
Å	Angstrom
AU	Arbitrary Unit
β	Beta
°C	Degree Celsius
g	Gram
h	Hour
J	Joule
k	Kilo
L	Liter
M	Mega
m	Milli
min	Minute
mol	Mole
n	Nano
ppm	Parts per million
Pa	Pascal
P	Pico

rpm	Revolutions per minute
s	Second
T	Temperature
θ	Theta
V	Volt
v	Volume
w	Weight
wt.%	Weight percentage

ANALISIS PERBANDINGAN HASIL BIOETANOL DAN KOMPONEN GULA YANG DIPEROLEH DARIPADA PELEPAH KELAPA SAWIT DI BAWAH RAWATAN PRAHIDROLISIS YANG BERBEZA

ABSTRAK

Permintaan bagi bahan bakar lebih mesra alam telah meningkat sejajar dengan pengurangan simpanan bahan bakar fosil. Malaysia merupakan pengeluar minyak sawit kedua terbesar di dunia, di mana sejumlah besar biomassa kelapa sawit dibuang tanpa sebarang penggunaan. Pelepah kelapa sawit (PKS) adalah salah satu biomassa kelapa sawit yang paling tidak digunakan walaupun mempunyai kemampuan menghasilkan tenaga yang tinggi. Oleh itu, kajian ini direka bagi menghasilkan bioetanol yang tinggi dari PKS yang menggunakan kaedah prarawatan yang mesra alam dan berdaya maju, iaitu prarawatan asid, autohidrolisis, alkali, alkali peroksida, dan alkali peroksida ditambah dengan prarawatan natrium klorit berasid (ASC). Keadaan tindak balas optimum yang diperlukan untuk mendapatkan hasil etanol maksimum dikaji bagi setiap kaedah prarawatan. Keadaan optimum adalah 1.5 b/i% H_2SO_4, 121 ° C, 90 min (bagi prarawatan asid), 180 ° C, 50 min (autohidrolisis), 3.5 b/i% NaOH, 121 ° C, 45 min (prarawatan alkali) , 3.5 b/i% NaOH dengan H_2O_2 (0.25 g/g biojisim), 121 ° C, 45 min (prarawatan alkali peroksida) dan untuk rawatan alkali peroksida-ASC, 0.4 g natrium klorit, 0.04 mL asid asetik g^{-1} biomassa kering pada suhu 80 ° C digunakan. Delignifikasi bagi kesemula kaedah prarawatan di atas adalah D_{auto} 36.31% < D_{asid} 47.50% < D_{alk} 51.67% < D_{peroxy} 64.54% dan < D_{ASC} 84.97%, masing-masing dengan G_{asid} 52.71% < G_{auto} 58.19% < G_{alk} 68.29% < G_{peroxy} 73.03% dan < G_{ASC} 84.64% pengolahan glukosa. Pengolahan etanol bagi PKS mentah, asid, autohidrolisis, alkali, alkali peroksida dan alkali peroksida-ASC masing-masing adalah E_{raw} 9.94% < E_{acid} 44.95% < E_{auto} 52.74% < E_{alk} 61.59% < E_{peroxy} 63.62%, dan < < E_{ASC} 75.11%, dengan indeks penghabluran CI_{raw} 56.81 % < CI_{auto} 60.65 % < CI_{acid} 62.80 % < CI_{alk} 63.69 % < CI_{peroxy} 67.89 % dan < CI_{ASC} 70.11%. Oleh yang demikian, delignifikasi, pengolahan glukosa dan etanol, dan nilai indeks penghabluran menerangkan bahawa kaedah prarawatan alkali peroksida-ASC paling sesuai bagi mencapai hasil etanol tertinggi dari PKS. Data FTIR dan SEM mengesahkan bahawa kaedah peroksida alkali-ASC menghasilkan jumlah serat selulosa tertinggi, menjadikannya lebih mudah bagi hidrolisis enzim.

COMPARATIVE ANALYSIS OF THE BIOETHANOL YIELD AND COMPONENTS OF SUGAR EXTRACTED FROM OIL PALM FROND UNDER DIFFERENT PREHYDROLYSIS TREATMENTS

ABSTRACT

The demand for greener fuels has increased with the depletion of fossil fuel deposits. Malaysia is the world's second largest palm oil producer, where a huge amount of oil palm biomass is discarded without proper utilisation. Oil palm frond (OPF) is one of the most underutilised oil palm biomass produced despite its high energy producing ability. Hence, this study was designed to produce a high bioethanol yield from OPF adopting eco-friendly and viable pretreatment methods, which are acid, autohydrolysis, alkaline, alkaline peroxide, and alkaline peroxide pretreatment coupled with acidified sodium chlorite (ASC) pretreatment. The optimum reaction conditions required to obtain the maximum ethanol yield were examined for each pretreatment method. Thus, optimum conditions were 1.5 w/v% H_2SO_4, 121 °C, 90 min (acid pretreatment), 180 °C, 50 min (autohydrolysis), 3.5 w/v% NaOH, 121 °C, 45 min (alkaline pretreatment), 3.5 w/v% NaOH with H_2O_2 (0.25 g g^{-1} of biomass), 121 °C, 45 min (alkaline peroxide pretreatment) and for alkaline peroxide-ASC treatment, 0.4 g of sodium chlorite, 0.04 mL of acetic acid g^{-1} dry biomass at 80 °C were used. The delignification of above pretreatment methods was D_{auto} 36.31 % < D_{acid} 47.50 % < D_{alk} 51.67 % < D_{peroxy} 64.54 % and < D_{ASC} 84.97 %, respectively with G_{acid} 52.71 % < G_{auto} 58.19 % < G_{alk} 68.29 % < G_{peroxy} 73.03 % and < G_{ASC} 84.64 % of glucose recovery. The ethanol recoveries for raw OPF, acid, autohydrolysed, alkaline, alkaline peroxide and alkaline peroxide-ASC pretreatments were E_{raw} 9.94 % < E_{acid}

44.95 % < E_{auto} 52.74 % < E_{alk} 61.59 % < E_{peroxy} 63.62 %, and << E_{ASC} 75.11 %, respectively with CI_{raw} 56.81 % < CI_{auto} 60.65 % < CI_{acid} 62.80 % < CI_{alk} 63.69 % < CI_{peroxy} 67.89 % and < CI_{ASC} 70.11 % of crystallinity index. Hence, delignification, glucose and ethanol recovery, and crystallinity index values affirmed that alkaline peroxide-ASC pretreatment method is most suited in attaining the highest ethanol yield from OPF. FTIR and SEM data confirmed that alkaline peroxide-ASC method exposed the highest amount of cellulose fibre, making them more accessible to enzyme hydrolysis.

CHAPTER 1

INTRODUCTION

1.1 Background study

Fossil fuel is the most abundant fuel type utilised for most of the energy needs for many decades. Due to its uncontrolled utilisation and with the increasing demand associated with the rising population and industrialisation. The natural fuel reservoirs are depleting exponentially. The generation of fossil fuel takes millions of years, whereas its combustion will need few seconds. The fossil fuel market is controlled by very few countries which lead to the fluctuation of prices rapidly. Moreover, the environmental effect of fossil fuel combustion outrages all of its benefits. Global warming is one significant influence of high fossil fuel consumption (Cardoso et al., 2017).

Thus, other renewable energy sources are emerging worldwide. Wind, solar, hydropower, geothermal, biomass, and biofuel have been utilised to produce a significant energy need (Azad et al., 2015). Bioethanol is one of the emerging renewable energy sources entering various industries due to its many advantages. However, bioethanol from edible plant sources decreases its interest as it depletes the food sources available for consumption (Carneiro et al., 2017). Simultaneously non-edible sources are widely used to produce sugar which is the precursor of bioethanol.

There are several advantages of bioethanol over fossil fuels. Firstly, it contains a higher amount of oxygen. Thus, it is capable of complete fuel combustion and has less carbon monoxide and unburned hydrocarbon emission with fewer additives. Second, the high octane number helps more efficient and cleaner engine functioning with fewer problems associated with knocking. The most significant advantage is the reduced emission of carbon dioxide which is the main greenhouse gas that leads to many climatic changes. The net carbon utilisation is nearly zero as the carbon emitted in combustion will be utilised for plant development as a source of photosynthesis. Also, the emission of sulfur and aromatic compounds, which causes many human health conditions, is relatively low (Demirbas, 2009).

In Malaysia agricultural industry significantly contributes to the country's economy. Among all the crops grown in Malaysia, the palm industry plays a crucial role as Malaysia is the second-largest palm oil producer globally (Parveez et al., 2021; Tang & Pantzaris, 2017; Yusoff et al., 2020). This is associated with producing many oil palm biomass wastes such as empty fruit bunches, chopped trunks, and dead fronds (Loh, 2017). However, this produced waste is not well managed and converted to valuable by-products. Many industries utilise this bio-waste to create paper, textile, souvenirs, and biofuel (Shuit et al., 2009). Oil palm empty fruit brunches are the highest utilised waste palm biomass. Oil palm fronds, though highly abounded, is still underutilised.

Accordingly, this research was carried out to utilise these waste palm fronds to produce bioethanol effectively (Derman et al., 2018). Five pretreatment methods have been occupied in this method: acid hydrolysis, alkaline hydrolysis, alkaline peroxide pretreatment, autohydrolysis and acidified sodium chlorite (ASC) pretreatment. The product obtained after each of the above pretreatment was hydrolysed and produced glucose which was fermented subsequently to produce ethanol.

1.2 Problem statement

Three main problems have been addressed in this study. First, the depletion of world fossil fuel reservoirs and the environmental effects associated with the consumption of fossil fuels demand the need for an alternative. However, biofuels derived from food sources affect global food scarcity. Hence, bioethanol production *via* greener approaches using non-edible lignocellulosic biomass has gained interest and needs further research to develop methods that can produce high bioethanol yield.

Next, Malaysia is the world's second-largest palm oil producer; therefore, massive amount of oil palm biomass are being generated annually. Out of all the biomass produced, oil palm fronds is the least utilized; so far, oil palm fronds is only being used as fertilizers. Therefore, they are piled to rot in plantation sites. When considering the mass of different oil palm biomass residues produced annually, oil palm fronds accounts for the highest amount. Even though it has an average gross calorific value, its energy-producing ability is the highest considering the total amount of mass-produced annually and yet is not utilized efficiently.

Finally, ethanol production from lignocellulose is tedious, as it requires a process known as pretreatment, which degrades the biomass and makes it vulnerable to chemical reactions. The pretreatment method should be selected according to the biomass used. Previous studies on the production of bioethanol from oil palm fronds is very limited. Therefore, the currently available pretreatment methods for the production of bioethanol from other oil palm biomass is less efficient for oil palm fronds and pretreatment with suitable conditions for higher bioethanol yield from oil palm fronds should be developed.

1.3 Research objectives

This research efficiently utilises the oil palm frond, a leftover in the palm oil production industry for bioethanol production *via* greener approaches. Furthermore, this study is designed to select the most efficient pretreatment method out of acid pretreatment, autohydrolysis, alkaline pretreatment, alkaline peroxide pretreatment and alkaline peroxide pretreatment coupled with acidified sodium chlorite (ASC) pretreatment that produces the highest bioethanol yield from *Saccharomyces cerevisiae*.

1. To analyse the proximate composition of oil palm fronds before and after each pretreatment method (acid pretreatment, autohydrolysis, alkaline pretreatment, alkaline peroxide pretreatment and alkaline peroxide pretreatment coupled with acidified sodium chlorite (ASC) pretreatment) in detecting the effect of pretreatment on the cellulose, hemicellulose and Klason lignin content.
2. To perform qualitative analysis such as SEM and FTIR in detecting the structure and main functional groups of the pretreated and untreated oil palm fronds and to detect the efficiency of each pretreatment process quantitatively through glucose and bioethanol recoveries.
3. To detect the optimum temperature, reaction time and concentration for each pretreatment method (acid pretreatment, autohydrolysis, alkaline pretreatment, alkaline peroxide pretreatment and alkaline peroxide pretreatment coupled with ASC pretreatment) to obtain the highest glucose and bioethanol yield.

1.4 Scope of the study

This study was designed to cover all aspects related to the objectives of the study. Initially, the palm fronds moisture, ash and extractive content were analysed using ASTM standard testing methods. Then, acid, autohydrolysis, alkaline, alkaline peroxide and acidified sodium chlorite pretreatment were performed to degrade the rigid lignocellulosic structure of the oil palm frond. In addition, the reaction time and temperature were varied to determine the optimum conditions for each pretreatment. Holocellulose, hemicellulose, cellulose and Klason lignin content of pretreated and untreated palm fronds is analysed according to TAPPI standard methods. The results were compared, and the efficacy of different pretreatment methods on the reduction of lignocellulosic content was analysed. Furthermore, the raw and pretreated biomass were tested to detect the functional groups present using FTIR. SEM detected the surface morphological changes that occurred during each pretreatment, and XRD detected the crystallinity changes of cellulose structure.

The pretreated biomass is subjected to both acid and enzymatic hydrolysis, and the glucose produced by both methods is compared to detect the most efficient method. The total soluble sugar content is analysed by UV whereas the different monosaccharide is detected by derivatisation with PMP (1-phenyl-3-methyl-5-pyrazolone) and HPLC is used to analyse the derivatised product. Subsequently, ethanol is produced by fermenting the sugars with the aid of *Saccharomyces cerevisiae* (Baker's yeast, Mauri-Pan strain), and the ethanol yield is detected by GC with a flame ionisation detector. Finally, the glucose recovery, pulp digestibility and ethanol yield are compared under different pretreatment conditions to detect the most efficient method for OPF.

CHAPTER 2

LITERATURE REVIEW

2.1 Biofuel

As the name implies, biofuels are types of fuel produced naturally from renewable sources, such as animal fat, plant biomass, and waste food and oils. These materials, either *via* biochemical or thermochemical processes, are converted to different forms of biofuels (Mohapatra et al., 2017). Globally, the leading biofuel producer is the United States which produces 46%, followed by Brazil, which makes 24%. The European Union, which accounts for 15%, and all the other countries produce around 15% of the total biofuel production (Cardoso et al., 2017).

Biofuel has 10-45% higher oxygen content than petroleum fuel, subsequently undergoes complete combustion. The plants take up the carbon dioxide produced during the combustion as the carbon source for photosynthesis. Consequently, the net carbon dioxide emission when considering biofuel derived from renewable sources is nearly zero. Carbon dioxide is the main contributor to the greenhouse effect produced by fossil fuels, making biofuel environmentally friendly compared to petroleum fuel. Besides, the biomasses are readily available or are mainly the waste products of other industries, such as the food industry and other industrial effluents, making biofuel comparatively cheaper and affordable and highly used in transportation, mainly in low-income farming communities (Demirbas, 2009).

Furthermore, the high octane number contributes to its clean-burning, making it safer to use in engines and reduces knocking. The United States and the European Union have already adopted flexible-fuel models in the newly produced cars. Around

50% of cars of General Motors, Ford, and Chrysler motor cars are designed for petroleum and biofuel blends (Ershov et al., 2016).

Biofuel can be broadly categorised into different groups according to the type of materials used and the chemical process utilised to produce the fuel. According to the type of biomass used, it can be categorised as primary biofuels and secondary biofuels. Bioethanol, biodiesel, and biogas are the three main types of biofuels classified according to the chemical process that is carried out. Bioethanol is the most abundant of all classes, estimated at 74%, biodiesel to be 24%, and only about 4% is biogas and bio-hydrogen.

2.2 Biofuel categorisation depending on the type of material

2.2.1 Primary biofuel

Primary Biofuels are fuels directly used to produce energy from burning, which does not include any additional processing steps. These are fundamental biofuels that have been used from the early days for domestic cooking and heating purposes. These include chopped wood, dried stick leaves and even landfill gasses (Rodionova et al., 2017). The main advantages of primary biofuels is that they are readily available, low cost, and no need for special processes before use. However, primary biofuels have few disadvantages, like higher greenhouse gas emissions, lower energy efficiency, and transportation problems (Nigam & Singh, 2011).

2.2.2 Secondary biofuel

Secondary biofuels are the biofuels that are derived from primary biofuels. These secondary biofuels are present in either solid-state as charcoal derived from wood or in the liquid state as bioethanol, biodiesel, or in the gaseous form such as biogas or biohydrogen (Doshi et al., 2016). Secondary fuels can be used for multiple ranges of applications, including transport and high-temperature industrial processes. The main advantages of secondary biofuels are cleaner burning, lower greenhouse gas emissions (unclean burning of primary fuels like chopped wood produces a lot of carbon, resulting in significant levels of air pollution), Higher energy efficiency, better waste utilization, which can be used for a wider range of applications, and also secondary biofuels are easy to transport (Nigam & Singh, 2011). The main disadvantages of secondary biofuels are the need for a secondary process to convert primary biofuels to secondary biofuels and secondary biofuels are more expensive than primary biofuels. (Nigam & Singh, 2011) These secondary biofuels can be further categorised into three generations based on their primary source and the technique used to produce the fuel (Azad et al., 2015).

2.2.2(a) First-generation biofuel

The first generation biofuel, as the name implies, was the first type of biofuel derived. The primary source is edible food and oil such as palm oil, sugar cane, sugar beet, vegetable oil, and animal fat. The production of bioethanol from these sources is comparatively easy as they contain a considerable amount of starch. The conversion of starch to sugar is more accessible than the conversion of cellulose to sugar. Also, biodiesel production can be done by directly transesterifying vegetable oils or animal fat (Rodionova et al., 2017). These types of biofuel are already commercialised in the USA, Brazil, and European Union (Joelsson et al., 2016; Rodionova et al., 2017). However, due to the utilisation of edible sources, there are many concerns about food scarcity with the increasing populations globally. Thus, researchers are moving on to develop fuels that are derived from non-edible food sources or food waste.

2.2.2(b) Second-generation biofuel

As an alternative to overcome the issues that arise due to the utilisation of food sources, the second generation of biofuels was derived from non-edible lignocellulosic biomasses (LCB). These structures are rigid due to their Klason lignin component, as a result they should undergo unique reactions known as pretreatment to break open the structure to access the cellulose component before producing sugar. However, the optimisation of these pretreatment methods requires a lot of time and expertise as the treatment method varies depending on the biomass used. Thus, the commercialisation of second-generation biofuels needs more time (Kumar et al., 2017; Rastogi & Shrivastava, 2017; Saini et al., 2016)

2.2.2(c) Third-generation biofuel

This is a new generation that is still under development that utilised diverse unicellular photosynthetic microalgae that are rapidly grown in saline water or in municipal/industrial wastewater, which harms the esthetic value of the environment (Serna et al., 2016). Hence, this type of biofuel acts as an alternative to overcome the problems associated with algal organisms. These organisms are either heterotrophic or autotrophic photosynthetic species. The autotrophic group uses carbon dioxide as the carbon source to produce carbohydrates. The produced carbohydrates can be converted to sugar and used to produce ethanol *via* fermentation. On the other hand, heterotrophic microalgae feed on organic molecules and convert them to lipids which can be later converted to biodiesel (Milano et al., 2016). Hence, either autotropic or heterotrophic microalgae can be grown in large ponds or bioreactors to produce bioethanol or biodiesel, depending on the need (Gambelli et al., 2017).

2.3 Biofuel categorisation according to the chemical process occupied

2.3.1 Biodiesel

The biofuel type produced from esterifying long-chain fatty acid present in vegetable oil or animal fat is biodiesel. It is economically feasible to produce biodiesel from cheaper oil such as palm oil or waste oil left over after cooking or industrial effluents. Like bioethanol, the USA is the largest biodiesel producer, accounting for 15.9% of the global biodiesel production. Brazil produces 12.9%, Germany 9.3%, France 7.9%, and all the other countries are responsible for making 53.8% of the total biodiesel production (Cardoso et al., 2017).

2.3.2 Biogas

The production of biohydrogen or biomethane via microbial fermentation falls under this category. Production of biohydrogen can follow either via dark or photo fermentation (Cheng & Zhu, 2016), and *Clostridium butyricum* can be used as the microorganism used for fermenting cellulose (Jiang et al., 2016). Biomethane can be produced from the anaerobic digestion of organic material (Budzianowski & Brodacka, 2017). Sugar cane, Corn, Wheat, Banana stem, and even parts of the palm tree are some biomasses capable of producing biohydrogen and biomethane.

2.3.3 Bioethanol

The biofuel type produced from fermenting sugar is known as bioethanol. The carbohydrates/starch present in food and food waste is mainly used to make the sugar that is later converted to bioethanol. However, recently the cellulose component of LCB is used to produce sugar as using food affects global food demand. The USA is the highest bioethanol producer, which annually produces around 50 billion liters. Second, on the list is Brazil which produces approximately 26 billion liters annually. The other countries that produce bioethanol include the European Union, Canada, Thailand, China, and India (Gupta & Verma, 2015; Sarris & Papanikolaou, 2016). The USA reported a mean bioethanol consumption of 10% in the year 2017 (Reboredo et al., 2017), while in the year 2013 in Brazil, 64% of the total vehicles were with Flexi fuel option (Laurini, 2017) as the government has made the blending of bioethanol with gasoline compulsory further the blending amount is increasing continually (Carneiro et al., 2017).

2.4 Bioethanol from lignocellulosic biomass

Initially, in the first generation of bioethanol was produced using edible plant sources. However, with the increase in global population and food scarcity, other non-edible food sources gained interest as the biomass used to produce bioethanol. Bioethanol production from lignocellulosic biomass is sustainable since lignocellulosic biomass is renewable and non-competitive with food crops. Also, compared with fossil fuels, lignocellulosic biomass is equally distributed around the globe (Bušić et al., 2018). Raw materials that contain lignocellulose can be classified into four groups: agricultural residues including straw, corn stover, bagasse and husks;

wood residues from the wood industry, including paper mills, sawmills and furniture manufacturing; dedicated energy crops including woody and herbaceous crops, primarily tall grasses; and municipal solid waste, including paper and other cellulosic materials (Nwakaire et al., 2013). Table 2.1 shows various feedstocks that can be used to make bioethanol via yeast-based fermentation and their relative production potential.

Table 2. 1: Different feedstock for yeast-based bioethanol production and their comparative production potential (Yusoff et al., 2015)

Feedstock	Potential ethanol yield (litre per dry tonne of feedstock)
Corn grain	470
Corn stover	428
Rice straw	416
Cotton gin trash	215
Forest thinnings	309
Hardwood sawdust	382
Bagasse	437
Mixed paper	440

2.4.1 Bioethanol from the fermentation process

There are three main types of microorganisms that are used to produce bioethanol via fermentation. *Saccharomyces* species, commonly known as yeast, bacteria of Zymomonas species, and mycelium (Yusoff et al., 2015). Out of all the above organisms, *Saccharomyces Cerevisiae* is the most commercial organism used n fermentation. Previous studies have shown that different strains show an altered pattern of utilising the substrate (Meneses et al., 2002). ATP is the man energy source of all living organisms, and glucose is the man precursor. Under aerobic or oxygen limiting conditions, the above-mentioned microorganisms convert the pyruvate produce from glycolysis to acetaldehyde and further convert to ethanol, producing two molecules of ATP and CO_2. This process is known as fermentation, which terminates the reaction when a high amount of ethanol is accumulated in the cells of the microorganisms (Maicas, 2020). The overall process which takes place during this reaction is shown in Figure 2.1.

Fermentation can be divided into alcoholic fermentation and non-alcoholic fermentation. Alcoholic fermentation is used in industries to produce ethanol in the form of alcoholic beverages such as wine and beer as well as to produce bioethanol and other chemicals. Not only in ethanol production but the fermentation is also occupied in different other industries such as bakery, coffee and chocolate industries which is known as non-alcoholic fermentation (Maicas, 2020)

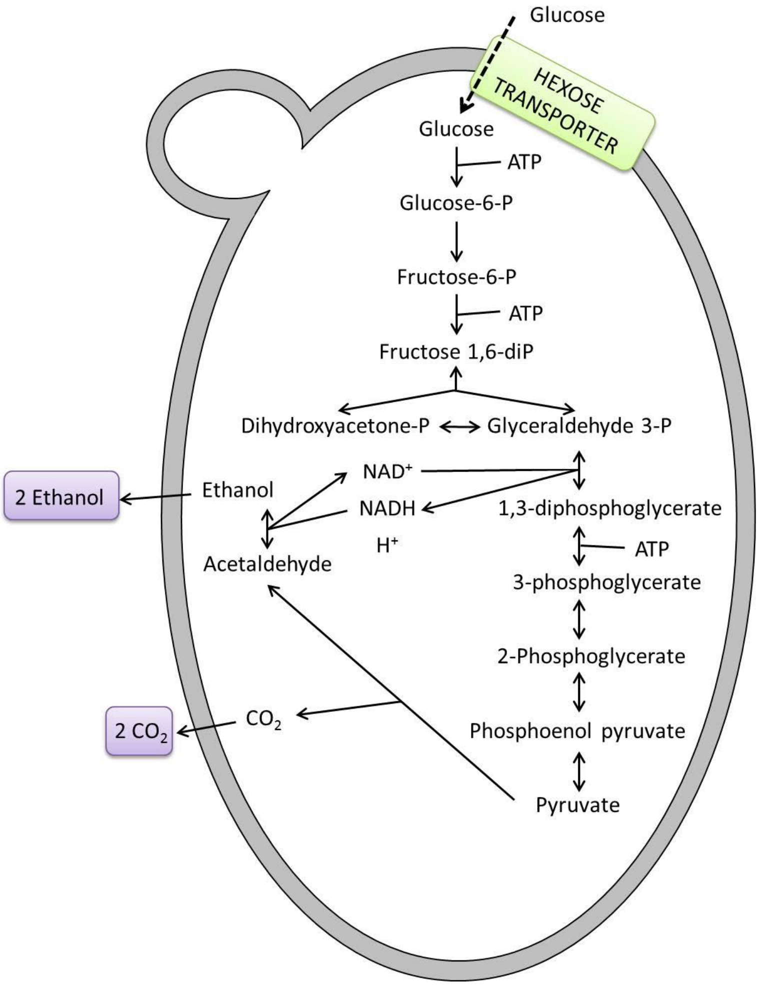

Figure 2 1: Fermentation process in yeast (Maicas, 2020).

2.4.2 Bioethanol from oil palm biomass

The type of oil palm biomass includes empty fruit bunches (EFB), oil palm trunk (OPT), palm oil mill effluent (POME) and oil palm frond (OPF), which contains considerably higher cellulose amount which can be used as a source for bioethanol production. Like other lignocellulose biomass, oil palm biomass contains higher Klason lignin content; due to higher Klason lignin content, oil palm biomass cannot be easily converted by biochemical processes due to biomass recalcitrance. Hence, pretreatment of oil palm biomass needs to carry out to remove the Klason lignin (Eom et al., 2015). Then the pretreated biomass is subjected to hydrolysis to convert the cellulose into monosaccharide sugar, and the sugars produced from the hydrolysis are used for the fermentation to produce bioethanol (Choowang, 2013).

A previous study done by Loh Soh et al. (2010) managed to produce 10.48 g L^{-1} of bioethanol from 50 g L^{-1} of EFB, which was initially pretreated with 1% NaOH followed by acid hydrolysis with 0.7% sulfuric acid and enzyme prior to the fermentation process with *Saccharomyces cerevisiae.* Another study that used empty fruit bunches as the source of biomass managed to produce 12.13 ± 0.99 g L^{-1} of bioethanol with a theoretical yield of 89.1% within 24 h (Bukhari et al., 2014). Before fermentation EFB was pretreated with 1.0% (v/v) dilute H_2SO_4 at 125°C for 90 min followed by 1% (w/v) NaOH at 100 °C for 60 min and subjected to enzymatic hydrolysis for 72 h. A study pretreated with 2% sodium hydroxide in hydrogen peroxide presoaking for 24 h at room temperature recovered 17.2 g L^{-1} of ethanol from oil palm frond (OPF) (Kumneadklang et al., 2015).

2.5 Lignocellulosic biomass

Lignocellulosic materials are found in three primary industries, agricultural wastes, residues of forest logging, and crops grown to produce biofuel. Out of the materials used to generate biofuels, lignocellulose is gaining tremendous interest due to its many good features. These renewable biomasses are highly available, cheaper, and produce lesser greenhouse gases during combustion. It comprises three main components, which are cellulose, hemicellulose, and Klason lignin. However, the amount of cellulose, hemicellulose and Klason lignin present differ significantly depending on different factors. The type of biomass (hardwood, softwood), the part of the tree (fruit, leaves, trunk, etc.), climatic/geometric conditions of the plant grown region, and the plant's age are some of them. Hardwood contains more cellulose, and parts such as leaves contain more hemicellulose (Bajpai, 2016). The bonding between these units makes it a rigid structure and difficult to release the cellulose moiety for ethanol production. Therefore, the structure is made chemically vulnerable by different pretreatments before glucose production (Bhatia et al., 2020).

2.5.1 Cellulose

The core of the lignocellulose is made up of 30-60% cellulose $(C_6H_{10}O_5)_n$ (Baruah et al., 2018), which is a homopolymer made up of 100 to 140,000 D-Glucose subunits, linked *via* β-1,4 glycosidic bonds (Abraham et al., 2020). This glycosidic bond can also be considered as an ether bond as it connects two carbon atoms via oxygen (Sasmal & Mohanty, 2018). As a result, 20 to 300 cellulose chains are boned via hydrogen bonds to form bundles of cellulose fiber (Figure 2.2). The presence of OH groups in the lateral parts of the subunit forms hydrogen bonds and makes the structure rigid. Thus, there is two forms crystalline and amorphous cellulose. The hydrogen bonds maintain the crystalline form. A large portion of the cellulose is in a well-packed crystalline state, and a small amount is in the disorganised amorphous form (Bajpai, 2016). Though cellulose is a hydrophilic compound due to its large structure, it is insoluble in water and requires a high/low pH medium for solubilisation (Baruah et al., 2018).

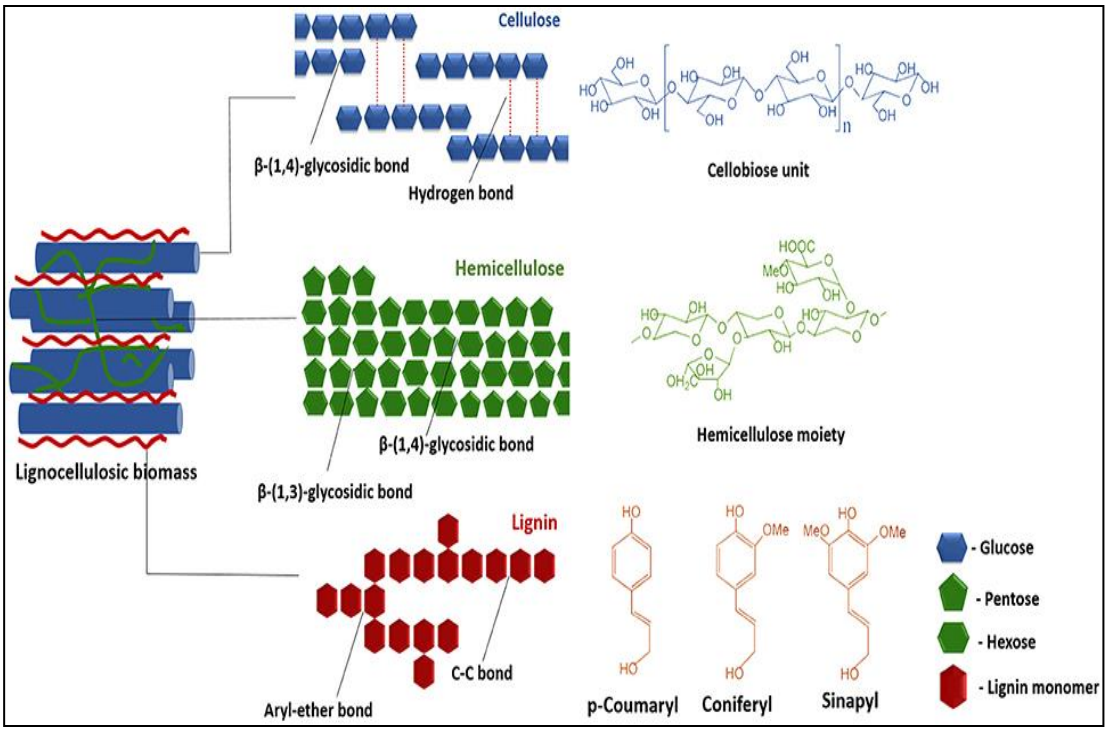

Figure 2 2: Schematic structure of lignocellulose (Baruah et al., 2018)

2.5.2 Hemicellulose

The cellulose bundles are coiled with hemicellulose, where the hemicellulose structure should be broken to digest cellulose first. Hemicellulose is a heteropolymer with 200 to 700 different monomer units (Abraham et al., 2020). These monomer units will be either different hexose, pentose, or xylans. Hemicellulose is the second-highest abundant form which contains about 20 to 50% of all cellulose, hemicellulose, and Klason lignin. The backbone of the hemicellulose will be short chains of either a homopolymer or a heteropolymer non-covalently linked *via* β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds (Figure 1) (Baruah et al., 2018).

Furthermore, hydroxyl groups present in the main chain will form an ester bond, with acetyl groups forming side chains (Sasmal & Mohanty, 2018). Hemicellulose consists of a loosely packed amorphous structure, hence the most vulnerable to chemical and thermal degradation and is highly utilised in many industries such as pharmaceuticals and cosmetics. However, the pretreatment conditions should be well optimised to minimise the production of hemicellulose derivatives, furfurals, and hydroxymethyl furfurals as they act as inhibitors in the fermentation reaction (Bajpai, 2016).

2.5.3 Klason lignin

Klason lignin is the large complex molecular form covering cellulose and hemicellulose, forming cross-links, making it rigid and most resistant to most biological and chemical reactions. Nearly 15 to 25% of lignocellulosic biomass is Klason lignin whereas, grasses contain the lowest Klason lignin, and softwood has the highest amount. Klason lignin is a heteropolymer that includes three main phenyl propionic alcohol monomer units, which are coniferyl alcohol (guaiacol propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). There are four different types of intra-molecular and inter-molecular bonds identified within Klason lignin: ether type bonds, ester bonds, hydrogen bonds, and carbon to carbon bonds. The monomer units of Klason lignin are connected 70% *via* ether type bonds and 30% *via* carbon-to-carbon bonds. These bonds can be present between alky and alky, aryl and aryl, or alkyl and aryl groups (Sasmal & Mohanty, 2018). This structure is hydrophobic and further acts as a barrier against decomposition. As a result, pretreatment is done to disrupt the Klason lignin to make cellulose and hemicellulose accessible for further conversions.

Klason lignin acts as a natural glue that sticks different components together, where it is used as a binder in the animal feed industry. Furthermore, it is also used as a plasticiser and a flavouring agent in food industries. However, despite the advantages, the sticking ability of Klason lignin has brought amount some adverse effects too. Some of them are interference with binding, nonproductive binding of cellulolytic enzymes to Klason lignin-carbohydrates complexes, and nonspecific adsorption of hydrolytic enzymes to “sticky” Klason lignin. Further, its derivatives may also be toxic to microorganisms needed for enzymatic hydrolysis and fermentation. Accordingly, it is always advisable to reduce the amount of Klason lignin present to a desirable level and utilise the removed Klason lignin to produce by-products.

Different types of bonds link the three main components of lignocellulose together. Hydrogen bonds are one type of bond that is identified as connectors of Klason lignin to cellulose and hemicellulose. Furthermore, ether bonds and covalent bonds are present between Klason lignin and polysaccharides. Besides, it is confirmed that ester bonds are present between Klason lignin and hemicellulose. Weaker hydrogen bonds between cellulose and hemicellulose are also identified since hemicellulose lacks peripheral primary alcohol groups (Sasmal & Mohanty, 2018).

2.6 Palm oil and oil palm wastes

2.6.1 Palm oil industry

The oil palm tree (*Elaeis guineensis*) was first introduced to Malaysia in 1870 as an ornamental plant. By 1960 the cultivated area started to increase exponentially, and by 2015 it was grown in 5.6 million hectares (Tang & Pantzaris, 2017). The first yield is harvested after 30 months of a plantation, and the grown tree has an economic level harvest for about 25 years. The fruit is the part that is utilised for palm oil extraction. A grown tree produces a bunch that weighs about 10-20 kg, which holds up to 2,000 fruits (Figure 2.3). The fruit has an internal kernel (seed) which is surrounded by a shell (endocarp) which is surrounded by another layer known as the mesocarp (Figure 2.4).

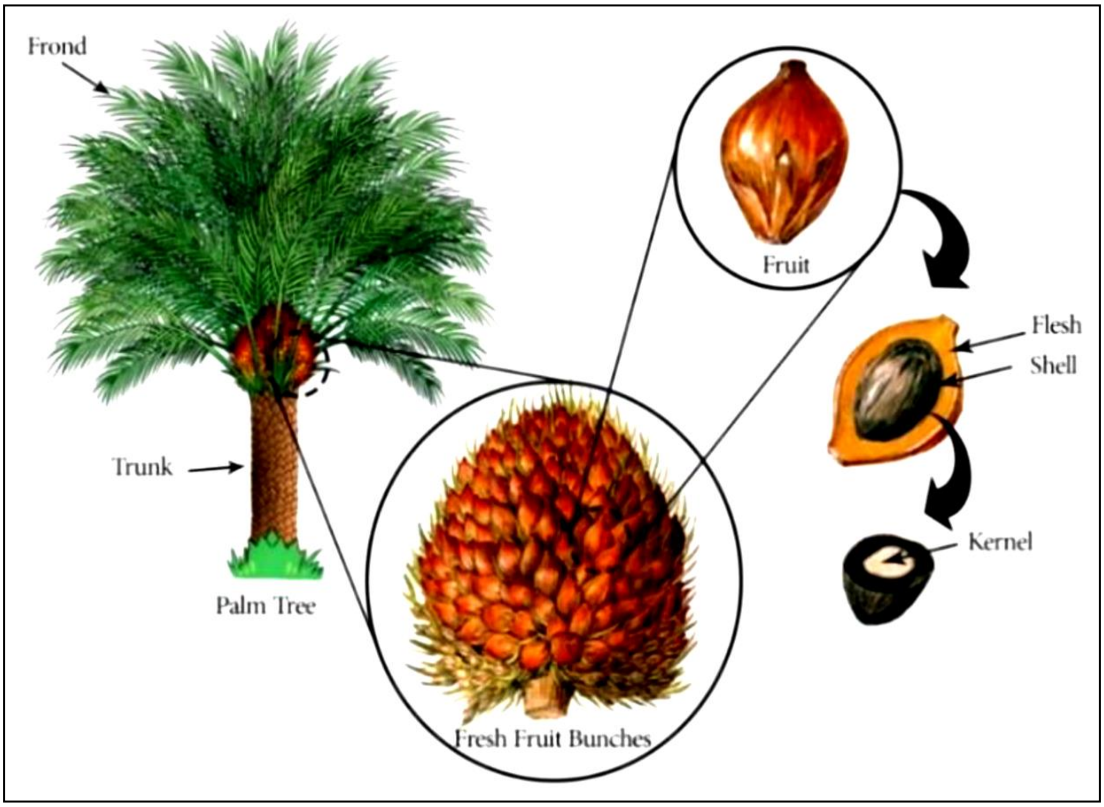

Figure 2 3: Parts of the palm tree (Babalghaith et al., 2020)

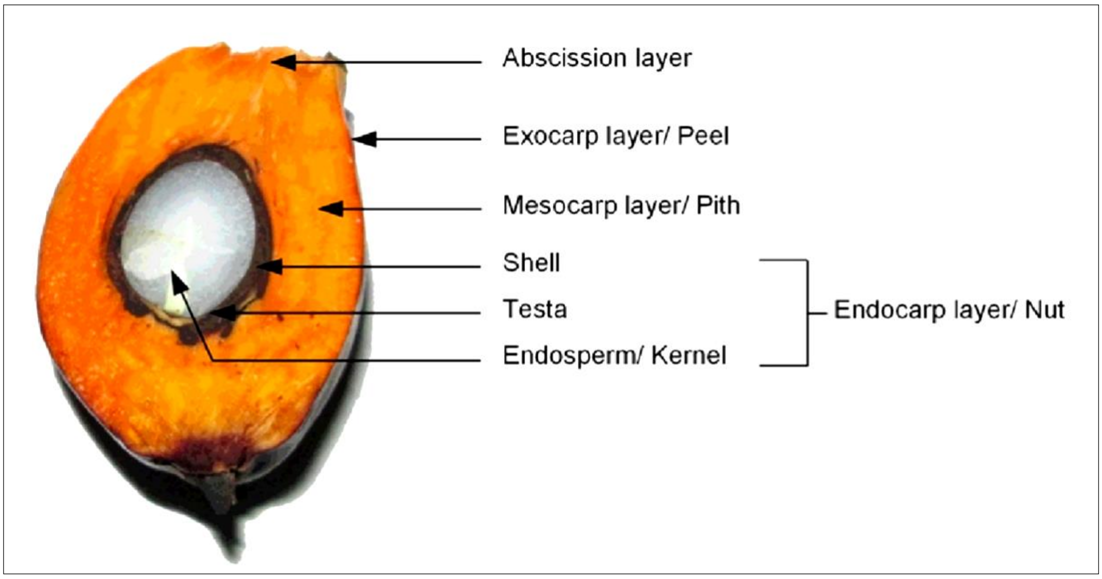

Figure 2 4: Cross-section of the palm fruit (Harun et al., 2016)

Both kernel and mesocarp can be used to produce palm oil. The kernel and mesocarp contain 50% and 49% oil, respectively. However, the composition of the oil extracted from different parts is different. The mesocarp oil contains the two most common fatty acids present in natural oils and fat: palmitic acid and oleic acid, and the saturation is 50%. On the other hand, the kernel oil contains more than 80% saturated lauric acid (Tang & Pantzaris, 2017).

With time, palm oil production in Malaysia has increased dramatically. In 1995, the total production was around 7.81 million tons, which reported its production as 19.96 million tons in 2015, which has increased by 12 million during the past two decades (Table 2.2).

Table 2. 2: Malaysian production of palm oil from 1995 to 2015 (Tang & Pantzaris, 2017)

Year	Tons
1995	7,810,546
2000	10,842,096
2005	14,961,654
2010	16,993,717
2013	19,216,459
2014	19,667,016
2015	19,961,581

Malaysia is currently the second-largest palm oil producer globally, which was initially the largest producer and was later overtaken by Indonesia. Table 2.3 depicts the production of palm oil in different countries from 1995 to 2015.

Table 2. 3: World production of palm oil (‘000 Tonnes) (Tang & Pantzaris, 2017) * (Food & Agriculture, 2020)

Producers	1995	2000	2005	2012	2013	2014	2015	2017*	2018*
Malaysia	7,810	10,842	14,962	18,785	19,216	19,667	19,961	19,919	19,516
Indonesia	4,220	7,050	14,100	26,900	28,500	30,800	33,400	37,965	40,567
Nigeria	660	740	800	940	970	1,010	940	1,040	1,130
Colombia	353	524	673	967	1,040	1,120	1,273	1,627	1,645
Côte d'Ivoire	300	278	290	405	415	420	522	483	450
Thailand	316	525	700	1,780	1,970	1,930	1,833	2,597	2,776
Papua new guinea	225	336	310	520	500	530	500	645	648
Ecuador	178	218	319	543	495	515	530	570	560
Others	1,149	1,354	1,798	3,043	3,207	3,331	3,599	3,842	4,176
Total	15,211	21,867	33,952	53,883	56,313	59,323	62,559	68,688	71,468

Palm oil is currently the most exported edible oil in the world. Table 2.4 and Table 2.5 display the palm oil and specifically palm kernel oil exported by different countries from 1995. Malaysia is also currently the second largest exporter of both palm oil and palm kernel oil globally.

Table 2. 4: World palm oil exports (‘000 Tons) (Tang & Pantzaris, 2017)

* (Food & Agriculture, 2020)

Major exporters	1995	2000	2005	2012	2013	2014	2015	2017*	2018*	2019*
Malaysia	6,513	9,081	13,445	17,575	18,147	17,278	17,454	13,689	13,841	15,201
Indonesia	1,856	4,139	10,436	19,094	21,471	22,080	26,548	27,308	27,893	25,626
Côte d'Ivoire	120	72	122	279	200	261	471	173	226	281
Papua new guinea	220	336	295	525	500	525	513	619	683	727
Others	1,485	1,391	2,213	3,338	3,657	3,452	3,244	5,842	6,125	5,981
Total	10,194	15,019	26,511	40,811	43,975	43,596	48,230	47,631	48,768	47,816

Table 2. 5: World palm kernel oil exports (‘000 Tons) (Tang & Pantzaris, 2017)

* (Food & Agriculture, 2020)

Major exporters	1995	2000	2005	2012	2013	2014	2015	2017*	2018*	2019*
Malaysia	391	520	851	1,085	1,171	1,111	1,067	572	644	758
Indonesia	311	579	1,043	1,669	1,722	1,628	1,890	1,631	1,772	1,429
Côte d'Ivoire	16	14	0	17	15	18	76	15	16	20
Papua new guinea	11	29	29	38	50	51	55	61	62	65
Nigeria	3	4	1	3	3	4	4	8	5	12
Others	64	74	175	253	281	313	218	481	505	433
Total	796	1,220	2,099	3,055	3,272	3,152	3,309	2,768	3,004	2,717

2.6.2 Oil palm biomass

Oil palm is the leading export agricultural crop that is grown in Malaysia. In the year 2015, the oil palm was cultivated across 5.64 million hectares in the country. The oil palm produced corresponds to only 10% of the dry mass-produced. The rest is discarded as waste despite its high economic value. Consequently, the highest biomass produced annually is subjected to the palm oil industry (Tang & Pantzaris, 2017).

Empty fruit bunches, mesocarp fibre, palm shell kernel, oil palm frond, and oil palm trunk are some of the direct biomass produced in the palm oil industry (Loh, 2017). Besides, palm oil mill effluents are made from the factories which produce palm oil. Figure 2.5 depicts the different forms of oil palm biomass available.

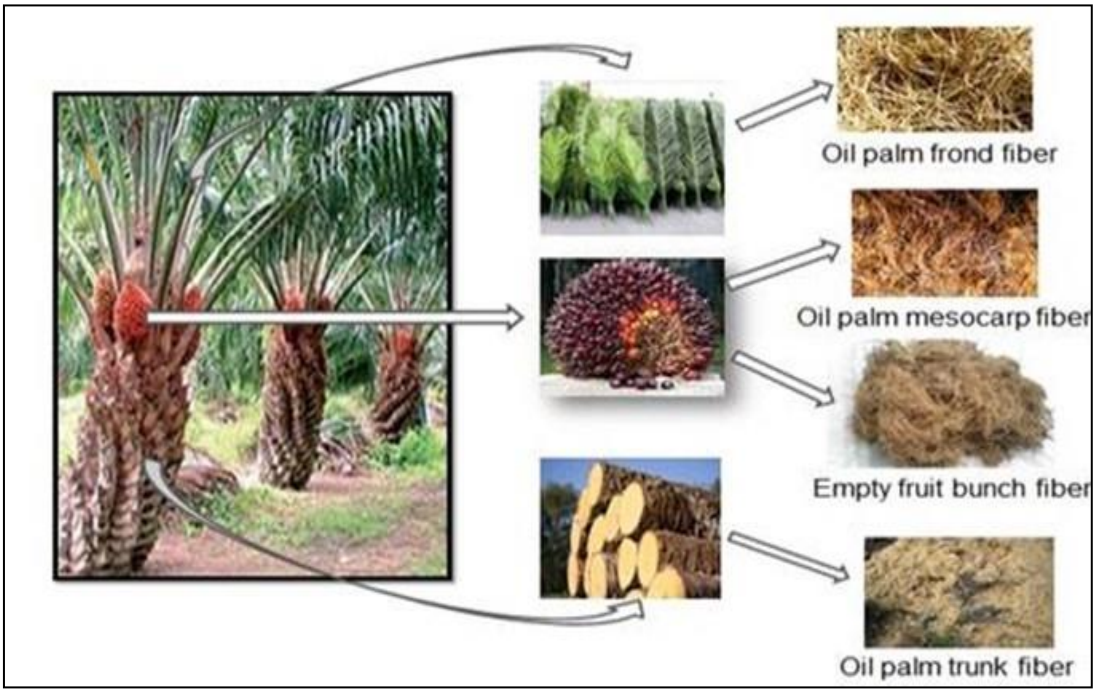

Figure 2 5: Oil palm biomass (Birnin-Yauri et al., 2016)