Lignocellulosic BIOFUEL
A Way Forward

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ABSTRACT

Economic dependency on fossil fuels and the resulting effects of its usage on the environment has placed considerable focus on utilizing biosugars from lignocellulosic biomass, the largest known renewable carbohydrate source as an alternative. Biosugars are derived from cellulose and hemicelluloses constituents; however these are in turn not readily accessible to enzymatic hydrolysis and hence requiring pretreatment, for extensive modification of the lignocellulosic structure. A number of pretreatment technologies are currently under development and tested at pilot scale. Hydrolysis of lignocellulose into biosugars requires a number of different cellulases and hemicellulases. The hydrolysis by cellulases is a sequential breakdown of the linear glucose chains, whereas hemicellulases must be capable of hydrolysing branched chains containing different sugars and functional groups. The technology for pretreatment and hydrolysis has been developed to an extent that is close to a commercially viable level. For example, processing of lignocelluloses at high substrate levels have become possible, all the while with improvements made on enzyme performances. In addition, the cost of enzymes has also been reduced. Nevertheless, a number of technical and scientific issues within pretreatment and hydrolysis remain to be solved and with significant expected improvements in yield and cost reductions, large-scale fermentation of lignocellulosic biomass is conceived to be possible. The concept of producing lignocellulosic biofuel, bioproducts and chemical through a biorefinery using lignocellulosic biomass had been around for 70 years or more. The use of renewable energy resources has become essential at a time when the focus is on global warming, carbon dioxide emission, security of energy supply, and reduction in consumption of fossil-based fuels. The recent interest in this

biorefinery concept is based on the mitigation of climate change by substituting the biomass energy for petroleum or other fossil-fuel energy. Thus the realization of biorefinery concept remains a challenge.

INTRODUCTION

Lignocellulosic material or lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose and lignin (Ibrahim, 2013). The major combustible component of non-food energy crops is cellulose, hemicellulose and followed by lignin. Currently, biomass is the most important source of renewable energy and the only renewable source of carbon. It can provide about 13% of total energy consumption worldwide (IEA Statistics, 2008). However, because much of this consumption concerns firewood-based heating and cooking, it cannot be considered as optimal use of lignocellulosic biomass resources. There remains considerable room for the development of lignocellulosic biomass value chains for the production of energy, chemicals, polymers and materials.

Lignocellulosic biomass is increasingly recognized as a valuable commodity, since it is an alternative to petroleum for the production of biofuels and chemicals. Even today, cellulose consumption is threefold higher than that of steel and is equal to that of cereals (Das and Singh, 2004), but its current uses are mainly restricted to the materials sector (wood-based and paper). The majority of lignocellulosic biomass is produced from agricultural, forestry, landscaping, and many other industries. The value increases from year to year, especially in a tropical country like Malaysia. Lignocellulosic biomass is currently being given much attention by researchers to be used as feedstock or raw material for many biological processes, since its major compositions are cellulose, hemicellulose and lignin. It can be burned directly to generate energy, or through biological and engineering processes, it can be converted into biofuels as shown in Figure 1, similar to fuel derived from petroleum.

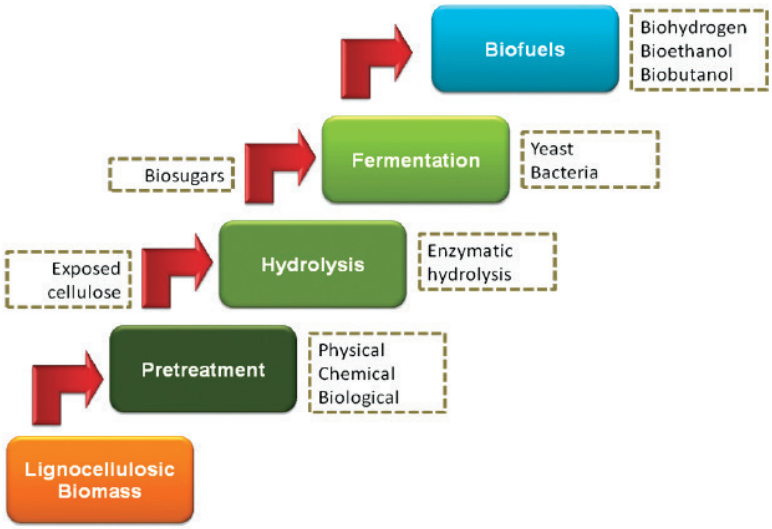

Figure 1 General process flow of lignocellulosic biomass to biofuels

Biofuel production is a recent development, which has gained significant attention due to the ever-dwindling supply of natural resources following our over-dependence on fossil fuels. This has stemmed a great deal of scientific research into the issue of alternative energy and biofuel has been seen as a potentially environmental and affordable way for us to reduce our dependency on fossil fuels. Up until recently, car manufacturers are highly reluctant to invest significantly in biofuel research for mainstream vehicles. This is because biofuel is to some extent an unproven technology - we know it works; just there is little research on the overall benefits of biofuel not only to consumers, but also to the planet. This has meant that until further research has been completed, many industries are reluctant to join in to develop biofuel into a sustainable and realistic form of energy.

Many people argue that the reason car manufacturers have started to adopt biofuel as a technology is mainly because of the pressure from governments across the globe due to the environmental impact that fossil fuels are having on the planet. Speaking from an environmental perspective, the rate of consumption for fossil fuel has risen exponentially in the past twenty years and as a result, we are now faced with the reality that fossil fuels such as petrol and diesel will run out within the next hundred years. Biofuels are a viable alternative to fossil fuels. Many varieties exist and they vary significantly. Some examples are that of biodiesel, which involves growing crops that contain high amounts of natural oil then through a process of hydrogenation or refining a compatible diesel substitute is created. The created biodiesel can then be mixed with mineral diesel to be used in any diesel-powered automobile.

A similar process in creating bio-petrol also exists, by fermenting crops producing sugar such as sugar cane. This creates natural ethanol, which can also be mixed with petrol to create hybrid biofuel capable for use in any petrol powered vehicle. However, ethanol's corrosively higher compared to petrol which presents a problem and as a result, it has limited use in aircrafts and boats. These are examples of first generation biofuel and due to their nature, they may or may not be long-term economically or environmentally viable. Arguments exist that both support the continued use of biofuel, however there is prove that they are not the miracle fuel we are waiting for.

The truth is that biofuel technology is still at its infancy. In the next twenty years, we anticipate to see biofuel research expand exponentially as we get closer to the day when fossil fuels are exhausted. Until then, continued reliance on fossil fuel will persist and we can only hope that biofuel as an alternative will be a reality before then.

LIGNOCELLULOSIC BIOMASS

In Malaysia, lignocellulosic biomass is mainly contributed by agricultural waste. Palm oil tree is the most planted plant with 4.92 million hectares (49200 km^2) of land area planted in 2011 (Malaysia Palm Oil Industry, 2011). The lignocellulosic biomass generated from palm oil plantations achieved up to 85.5% of total biomass production in 2006 (Shuit *et al.*, 2009). The high production of plant biomass in Malaysia was due to the high sunlight intensity over time and high rainfall per year. Agriculture sector contributed about 41% of the Malaysia Growth National Income (GNI) in 2010 (Agensi Inovasi Malaysia, 2011).

In the year of 2006, approximately 51.2 million tonnes of oil palm biomass was produced in Malaysia. The number represented 15.8 million tonnes of oil palm empty fruit bunch (OPEFB), 12.9 million tonnes of oil palm frond (OPF), 9.6 million tonnes of mesocarp fiber, 8.2 million tonnes of oil palm trunk (OPT) and 4.7 million tonnes of oil palm shell (Malaysia Palm Oil Board, 2006). These numbers have increased greatly in the past few years. In 2010, the total oil palm biomass recorded was almost 80 million tonnes. This value is estimated to increase up to 85-110 million tonnes in 2020 (Agensi Inovasi Malaysia, 2011). The lignocellulosic biomass generated from the palm oil industry is shown in Figure 2.

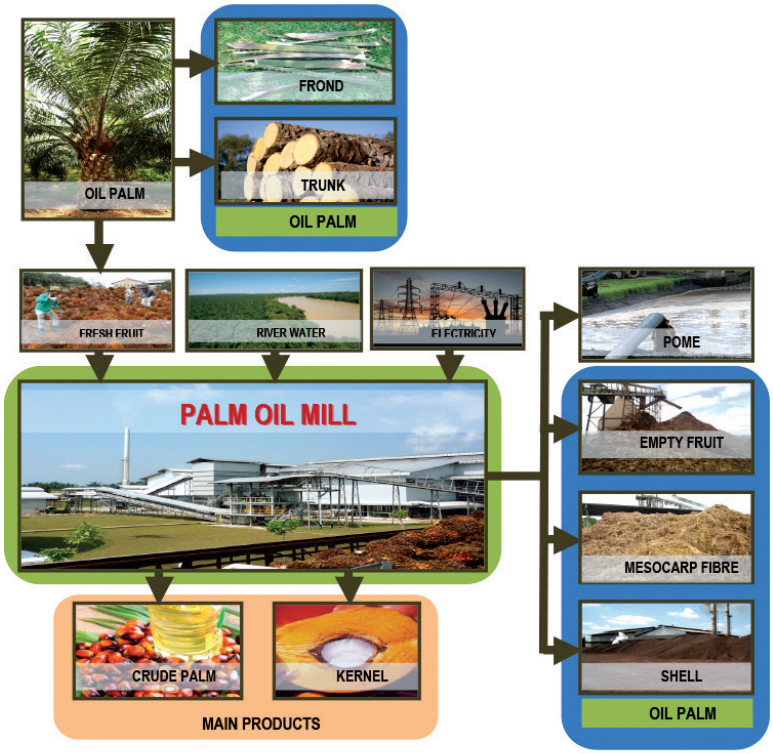

Figure 2 Oil palm biomass generated in the palm oil plantation and palm oil mill
(Source: EB Research Report, 2013; Ibrahim, 2013)

Besides palm oil industry, Malaysia also produces other types of lignocellulosic biomass such as sago biomass and rice biomass. Sago biomass is produced from the processing of sago starch. Malaysia is recognized as one of the world's biggest exporters of sago starch, which is currently the largest sago-growing areas, with export over the past 10 years being between 55,000-65,000 tonnes/ year of starch mainly to Peninsular Malaysia, Japan, Singapore, and other countries. The extraction of sago starch involves debarking,

rasping, sieving, settling washing, and drying (Awg-Adeni *et al.*, 2010). During the extraction of sago starch, three types of waste have been produced; sago bark, sago *hampas* and sago wastewater. Figure 3 shows the flowchart of sago starch processing. However, those wastes are not yet well utilized and are disposed into the nearby river without proper treatment hence causing pollution to that area. Considering the value of this sago biomass and the importance of conserving the environment, research efforts has continuously been conducted to properly utilize these wastes. Sago *hampas* contains about 50% of starch with the remaining constituents being lignin, cellulose and hemicelluloses (Abd-Aziz, 2002; Abd-Aziz *et al.,* 2010). The starch can be hydrolyzed using Dextrozyme (an amylase) producing sago *hampas* hydrolysate in a liquid form (Shahrim, 2006; Shahrim *et al.,* 2008) and sago pith residue (SPR), the remaining solid residue (Linggang, 2013). This lignocellulosic material consists of about 60% of cellulose and hemicellulose with lignin, ash and other component materials representing the remaining components (Jenol *et al.*, 2014; Linggang *et al.*, 2012).

Paddy is also an important crop in Malaysia and it is vital for the nation's food safety (Fatimah *et al*., 2007). In Malaysia, the total paddy production increased from 2,257 tonnes in 2003 to 2,384 tonnes in 2008 while the average yield of paddy increased from 3,360 kg/ha in 2003 to 3,556 kg/ha in 2008. Land utilization for paddy production is currently at 674,928 hectares with 76% being in Peninsular Malaysia (515,657 ha) while Sarawak and Sabah accounts for 18% (118,919 ha) and 6% (40,352 ha) of the total hectares, respectively (Ramli *et al*., 2012). Apart from providing the country's staple food, the rice industry has also generated stable income for the country.

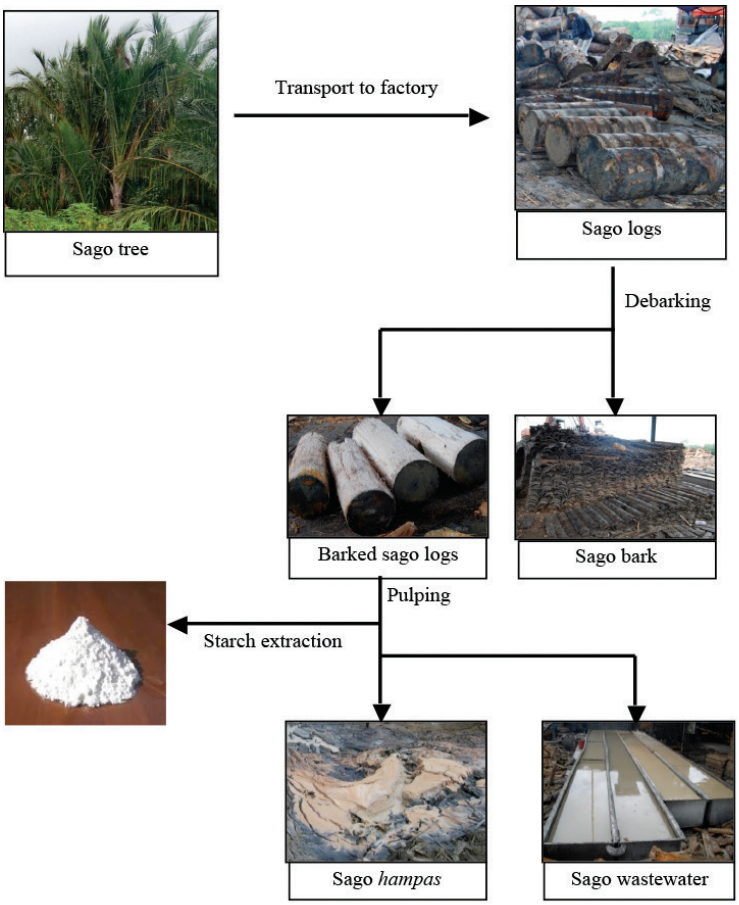

Figure 3 The schematic flowchart of sago starch extraction process
(Source: Awg-Adeni *et al.*, 2010)

Rice processing has always contributed to a large amount of rice by-products. During the processing of paddy rice, few parts are produced to be consumed by human while the by-products are usually disregarded or utilized for other purposes. The by-products produced after processing of paddy rice include bran, husk, straw,

polished rice and broken rice as shown in Figure 4. Rice straw is one of the most abundant agriculture residues produced from rice processing. The rice plants (dry weight) are composed of about 50% of rice straw, with a significant variation from 40 to 60% according to the cultivar and cultivation method. For every tonne of grain harvested, about 1.35 tonnes of rice straw remain in the field. Rice straw has high potential as a source of lignocellulosic biomass because of its high yield per hectare. The amount of recoverable straw depends of the method of reaping and harvesting and on the condition of the field (wet or dry) and crop (lodged or not). The average net production of dry straw is about 5.6-6.7 t/ha (2.5-3.0 tonnes/acre) (Mohamad Remli, 2014).

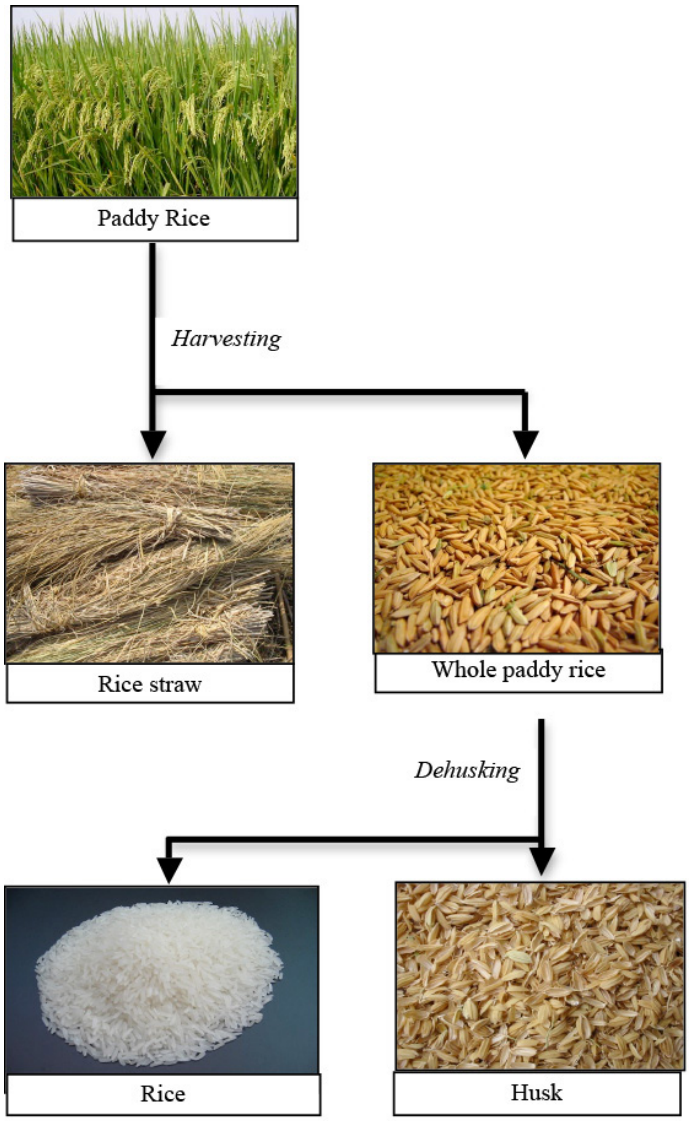

Figure 4 The schematic flowchart of paddy processing in rice industry
(Source: Mohamad Remli, 2014)

Lignocellulosic biomass is a plant-based material composed of lignin, cellulose and hemicellulose. This class of biomass includes wood and fibrous materials from organic sources, agricultural wastes, organic municipal wastes and organic industrial wastes (Abd-Aziz and Hassan, 2009). On average, lignocellulosic biomass is composed of 38-50% of cellulose, 23-32% of hemicellulose and 15-25% of lignin. Cellulose is physically associated with hemicellulose, and physically and chemically associated with lignin (Ibrahim, 2013). Basically, the individual cellulose molecules are linked together to form elementary microfibrils, of which in turn are aggregated by intermolecular hydrogen bonding into larger subunits called fibrils. The microfibrils contain alternating phases of highly ordered (crystalline) and randomly oriented (amorphous) cellulose embedded in a matrix of hemicellulose (Bahrin, 2012). The cellulose and hemicellulose fractions are covered in an amorphous layer of lignin (Astimar *et al.*, 2002). The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis process (Razak *et al.*, 2012). The ratio of cellulose, hemicellulose and lignin within the polymer varies between different plants, wood tissues and cell wall layers (Rubin, 2008). Figure 5 shows the organization of cellulose, hemicellulose and lignin in the microfibril structures of plant cell wall.

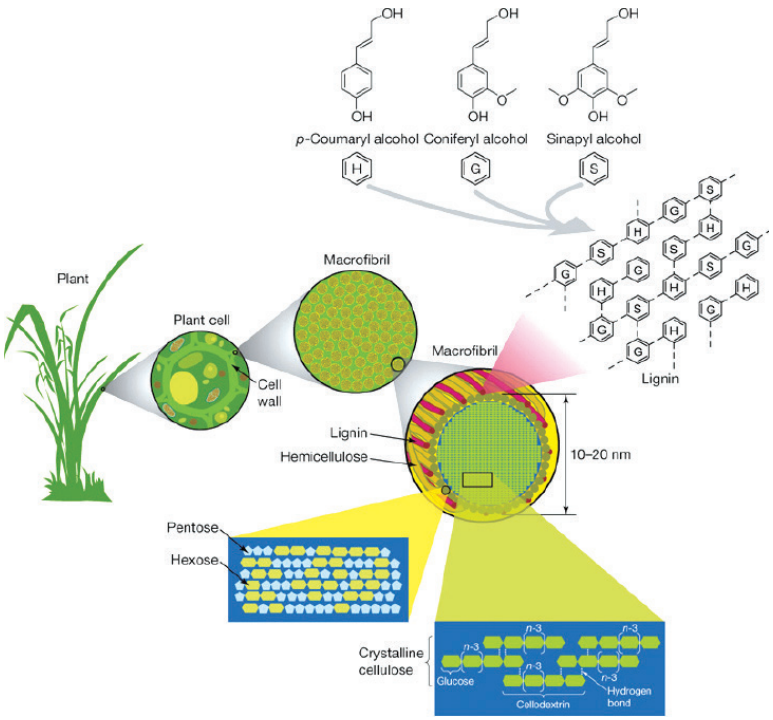

Figure 5 Structure of lignocellulosic material
(Source: Rubin, 2008)

a. *Cellulose:* Cellulose is an organic polysaccharide consisting of a linear chain of several hundreds to over nine thousand β(1→4) linked D-glucose $(C_6H_{10}O_5)$n units. Cellulose, a fibrous, tough, water-insoluble substance, is found in the cell walls of plants, particularly in the stalks, stems, trunks and all the woody portions of the plant body (Nelson *et al.*, 2008). Cellulose comprises 40-60% of the dry weight of plant material and this composition is dependent on the plant species and their parts (Ibrahim, 2013).

Shaw (2008) reported that binding of wood material during hot pressing/densification is mainly dependent on the transition of cellulose into the amorphous state. According to Bahrin (2012), due to the semi-crystalline structure, hydrogen bonded cellulose cannot be dissolved easily in conventional solvents, and it cannot be melted before it burns; hence, cellulose itself is not a suitable adhesive. This can be overcome by breaking the hydrogen bonds, thus making the cellulose molecule more flexible. Cellulose requires a temperature of 320°C and pressure of 25 MPa to become amorphous in water.

b. *Hemicellulose:* Hemicellulose is made of several heteropolymers (matrix polysaccharides) present in almost all plant cell walls along with cellulose. While cellulose is crystalline, strong, and resistant to hydrolysis; hemicellulose has a random, amorphous structure with less strength. Hemicellulose is a polysaccharide related to cellulose and comprises 20-40% of the biomass of most plants (Ibrahim, 2013). In contrast to cellulose, hemicellulose is derived from several sugars in addition to glucose, including especially xylose but also mannose, galactose, rhamnose and arabinose (Shambe and Kennedy, 1985). Branching in hemicellulose produces an amorphous structure that is more easily hydrolyzed than cellulose (Shaw, 2008). Also, hemicellulose can be dissolved in strong alkali solutions. Hemicellulose provides structural integrity to the cell. Some researchers believe that natural bonding may occur due to the adhesive properties of degraded hemicellulose (Bhattacharya *et al.*, 1989).

c. *Lignin:* Lignin is a complex chemical compound most commonly derived from wood and is an integral part of the cell walls of plants (Zandersons *et al.,* 2004). The compound has several unusual properties as a biopolymer, not the least its heterogeneity in lacking a defined primary structure. Lignin fills the spaces in the cell wall between cellulose and hemicellulose. It is covalently linked to hemicellulose and thereby crosslinks different plant polysaccharides, conferring mechanical strength to the cell wall and consequently to the whole plant structure (Bahrin, 2012; Razak, 2013).

Lignin acts as a binder for the cellulose fibres. This component melts at temperatures above 140°C (Bahrin *et al.*, 2012a), therefore it exhibits thermosetting properties. Lignin is the component that permits adhesion in the wood structure, and is a rigidifying and bulking agent. The adhesive properties of thermally softened lignin are thought to contribute considerably to the strength characteristics of briquettes made of lignocellulosic materials (Shaw, 2008).

THE KEY TO EFFECTIVE ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSIC BIOMASS

Enzymes play a critical role in the conversion of lignocellulosic waste into fuels and chemicals, but the high cost of these enzymes presents a significant barrier to commercialization. In the simplest terms, the cost is a function of the large amount of enzyme protein required to break down polymeric sugars in cellulose and hemicellulose to fermentable monomers. Significant effort has been expended to reduce this by focusing on improving the efficiency of known enzymes, identification of new, more active enzymes, or creating mixture of enzyme cocktail in order to improve the

hydrolysis efficiency for selected pretreated substrates, and therefore minimizing production costs.

The enzymatic process is regarded as the most attractive way to degrade cellulose to glucose (Ibrahim *et al.,* 2013; Razak *et al.,* 2012; Yu and Zhang, 2004). However, enzyme-catalysed conversion of cellulose to glucose is very slow unless the biomass has been subjected to some form of pretreatment, as native cellulose is well protected by a matrix of hemicellulose and lignin (Razak *et al.,* 2012; Bahrin *et al.,* 2012a). Pretreatment of the raw material is perhaps the single most crucial step as it has a large impact on all the other steps in the process, e.g. enzymatic hydrolysis, fermentation, downstream processing and wastewater handling, in terms of digestibility of the cellulose, fermentation toxicity, stirring power requirements, energy demand in the downstream processes and wastewater treatment demands.

An effective pretreatment should have a number of features that include (Galbe and Zacchi, 2007):

i. High recovery of all carbohydrates in the system.
ii. High digestibility of the cellulose in the subsequent enzymatic hydrolysis.
iii. Very limited amounts of sugar and lignin degradation products or zero residues. The pretreatment liquid should be possible to ferment without detoxification.
iv. Pretreated liquid fraction containing high solids concentration as well as high concentrations of liberated sugars.
v. Pretreated residues have low energy demand or can be presented in a way so that the energy can be reused in other process steps as secondary heat.
vi. Low capital and operational cost.

The above features have been mentioned by Bahrin (2012) and Razak (2013) on the importance of pretreatment to open up, alter or remove the lignin structure so that the internal cellulose is exposed for cellulase action. The action of cellulase on pretreated lignocellulosic biomass has been explained by Ibrahim (2013) and Linggang (2013), where the efficiency is mostly dependent on the yield of biosugars produced with low amounts of cellulase used. Additional positive features are present if hemicellulose sugars are obtained in the liquid as monomer sugars, this would help to avoid the use of hemicellulases, and/or if the lignin without being oxidized is separated from the cellulose, this would alleviate the unproductive binding of cellulases on lignin in the enzymatic hydrolysis step.

Assessment of pretreatment is usually done by using some of (or a combination of) the following methods:

i. Analysis of the content of sugars liberated during pretreatment to the liquid as a combination of monomer and oligomer sugars, as well as analysis of the carbohydrate content of the water-insoluble solids. This gives the total recovery of carbohydrates in the pretreatment step (Linggang, 2013).

ii. Enzymatic hydrolysis of the water insoluble solid, either washed or non-washed.

iii. Fermentation of the pretreatment liquid to assess inhibition of the fermenting microorganism (Razak, 2013).

iv. Simultaneous saccharification and fermentation (SSF) of either the whole slurry or the washed water insoluble solid (Ibrahim, 2013).

The enzymatic hydrolysis (in i and iv) is performed using cellulases, i.e. a mixture of various cellobiohydrolases and endoglucanases supplemented with β-glucosidase. The latter is not a cellulase as it only cleaves cellobiose into two glucose molecules.

It has, however, a very important role in hydrolysis since cellobiose is an end-product inhibitor of many cellulases (Ibrahim *et al.,* 2013; Ibrahim, 2013). On the other hand, β-glucosidase is also inhibited by glucose (Ibrahim *et al.,* 2013; Linggang *et al.,* 2012). Since the enzymes are inhibited by the end products, the build-up of any of these products affects cellulose hydrolysis negatively. The maximum cellulase activity for most fungus-derived cellulases and β-glucosidase occurs at 50±5°C and a pH of 4.0-5.0. However, the optimal conditions for enzymatic hydrolysis change with the hydrolysis residence time and are also dependent on the source of the enzymes and types of substrate (Ibrahim *et al.,* 2013; Razak *et al.,* 2012; Linggang *et al.*, 2012). Although the properties of the cellulase enzyme complex has a significant effect on how effectively a lignocellulosic material will be hydrolyzed, it is the biomass pretreatment and the intrinsic structure/composition of the substrate itself that are primarily responsible for its subsequent hydrolysis by cellulases. It is apparent that in a sequential series of events, the conditions employed in the chosen pretreatment will affect various substrate characteristics, which in turn govern the susceptibility of the substrate to be hydrolyzed by cellulase and subsequent fermentation of the released biosugars.

Choosing the appropriate pretreatment for a particular biomass feedstock is frequently a compromise between minimizing the degradation of the hemicellulose and cellulose components while maximizing the ease of hydrolysis of the cellulosic substrate. The digestibility of pretreated lignocellulosic substrates is further complicated by the lignin-hemicellulose matrix in which cellulose is tightly embedded. Pretreatment conditions can be tailored to create either solid or solid/liquid substrates with varying levels of cellulose, hemicellulose and lignin. It is apparent that lignin affects enzymatic hydrolysis by blocking cellulose and by chemical

interactions facilitated by its hydrophobic surface properties and various functional groups. The role of hemicellulose is less obvious although there is good evidence to support the action of hemicellulose as a barrier restricting access to cellulases. In the past, many investigators have attributed to enhance the enzymatic hydrolysis performance of a particular pretreatment to change in the proportion of the lignin, hemicellulose and cellulose in the substrate. However, it is important to advance this conclusion one step further as it is likely that decreases in lignin and hemicellulose content that occur as a result of pretreatment also affect the physical properties of the cellulosic component, such as its crystallinity, the degree of polymerization and the surface area of the substrate accessible to cellulases. Therefore, the search for efficient pretreatment methods is compulsory in order to hydrolyze lignocellulosic biomass into biosugars prior to biofuel production.

THE SEARCH FOR EFFICIENT PRETREATMENT METHODS

The protective structure of lignin that covers the internal cellulose and hemicellulose has brought forward the challenge to convert this polymer into simple sugar monomers or so called biosugars. Thus, pretreatment has been proposed to alter the lignocellulosic structure to be accessible by cellulase (Bahrin, 2012). Figure 6 illustrates the effect of pretreatment process applied to the lignocellulosic biomass. Pretreatment is considered as one of the most expensive processing steps in the conversion of cellulosic polymer into sugar monomers (Alvira *et al.*, 2010). An effective pretreatment is determined based on several criteria; avoiding size reduction, preserving hemicellulose fractions, limiting formation of inhibitors, minimizing energy input and being cost-effective (National Research Council, 1999). The hydrolysis percentage of

cellulose into biosugars is also considered as a criterion of effective pretreatment (Razak, 2013). The pretreatment methods have generally been divided into three categories; physical, chemical and biological pretreatment. Combination of pretreatment by conjugating two or more pretreatment methods from the same or different categories are also commonly practiced (McMillan, 1994; Mohamad Remli *et al.*, 2014).

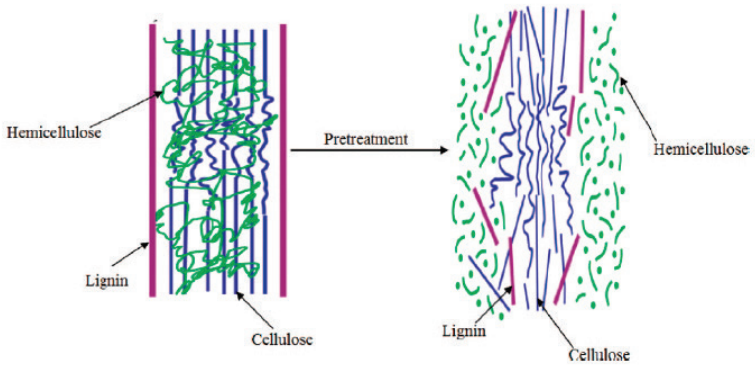

Figure 6 Schematic presentation of the effect of pretreatment on lignocellulosic biomass
(Source: Mosier *et al.,* 2005)

Physical Pretreatment

Physical pretreatment usually applies to any physical approaches done to alter the structure of lignocellulosic material by the use of steam explosion, hot water, mechanical comminution and/or energy radiation (Bahrin, 2012). Steam explosion is one of the most common pretreatment methods applied for lignocellulosic biomass. It uses water steam with temperatures of 160-270°C for several seconds to a few minutes and leads to physiochemical changes of hemicellulose and lignin (Shamsudin, 2013). Figure 7 shows the effect of this pretreatment on pressed and shredded OPEFB. Flake

and spherical geometry of OPEFB particles were found from the treated OPEFB at 140°C and 180°C, respectively. Most of the treated OPEFB were mixtures of various sizes and shapes when treated at more than 180°C. OPEFB with cylinder-like geometry has resulted in the highest aspect ratio among other geometry, making it more favourable for bioconversion process (Bahrin *et al.,* 2011; Bahrin *et al.,* 2012a).

Figure 7 Observation of OPEFB particle geometry; spherical shape (a), flake-like (b), and cylinder-like (c), after treated with 210°C of superheated treatment
(Source: Bahrin *et al.,* 2012a)

Steam explosion is considered as one of only limited low cost lignocellulosic pretreatment method that had been introduced to the pilot scale and commercial application (Zheng *et al.*, 2009). However, low hemicellulose sugar yield has been one of the disadvantages of this treatment. Alternatively, liquid hot water (LHW) treatment method has attracted interest as one of the most effective lignocellulosic pretreatment. This pretreatment has the potential of cellulose digestibility, pentose recovery, biosugars extraction and has little or no inhibitory effect towards microorganism fermentation (van Walsum *et al.*, 1996). Pressure is utilized to maintain the water condition at liquid state with elevated temperature. Another common practice of physical pretreatment is mechanical comminution. This method involves the breaking down of the lignocellulosic biomass into small particle sizes and

thus enhancing the surface area to be accessed by the enzyme. It is used by any mechanical milling, grinding or chipping machine. However, this method requires high energy consumption to rotate the milling or grinding motor and is also time consuming, thus making the whole process expensive.

Chemical Pretreatment

Chemical pretreatment is the most studied form of lignocellulosic pretreatment method when compared to other categories. This treatment originated from the paper industry to delignify the cellulosic components in order to produce high quality of paper (Fan *et al.*, 1982). Acid and alkaline pretreatments are the most common method applied under this category. Acid hydrolysis of lignocellulosic biomass is usually performed using either sulphuric acid (H_2SO_4) or hydrochloric acid (HCl) that has strong digesting capability to break the polysaccharide bonds of cellulosic material into monomers. Although this method is effective and powerful enough for cellulose hydrolysis, acid is however toxic, corrosive, hazardous and not environmental friendly.

The sugars produced by acid hydrolysis also require further treatment to remove inhibitory components prior to microorganism fermentation as has been done by Zainudin *et al.* (2012) and Zainudin (2009) using phase-separation system to recover glucose from acid-hydrolysed OPEFB. The lignocellulosic digestion by acid has also been tested on OPDC by Razak *et al.* (2012), but it seems not to be attractive as compared to alkaline pretreatment. This is because acid treatment hydrolyzes cellulose into biosugars while alkaline treatment solubilises the lignin material into small droplets (Umi Kalsom *et al.,* 1997), thus exposing the internal cellulose structure to be hydrolysed by cellulases. Therefore, alkaline treatment is the most studied pretreatment for lignocellulosic

biomass that employs various bases including sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), potassium hydroxide (KOH), ammonium hydroxide (NH_4OH) and other related alkaline chemicals (Zheng *et al.*, 2009). This treatment causes the swelling effect to lignocellulosic biomass, in turn causing depolymerization and decrease in crystallinity by disrupting the lignin structure and subsequently increasing the exposure of the internal surface and making it accessible to the enzyme digestion (Ibrahim, 2013) as shown in Figure 8. Considering the waste generated from this pretreatment, alkaline hydrolysate (black liquor of the solubilized lignin) has been tested for the production of biovanillin (Aanifah, 2013; Aanifah *et al.*, 2014) and its absorption has been done by Abu Samah *et al*. (2013).

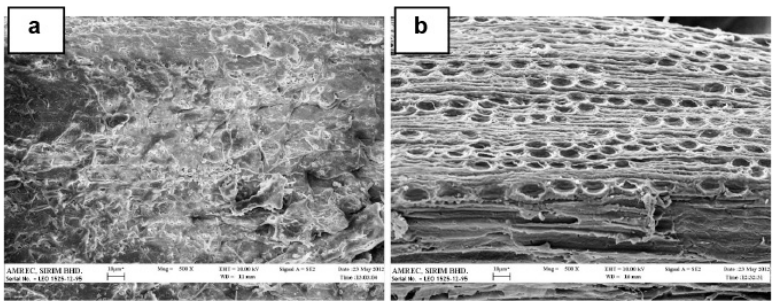

Figure 8 Untreated OPEFB (a) and pretreated OPEFB (b) under 500× magnification of scanning electron microscope (SEM) which shows the exposure of cellulose structure after treated with 2% NaOH with autoclaved
(Source: Ibrahim, 2013)

Biological Pretreatment

Biological pretreatment is the degradation of lignocellulosic material by microorganisms mainly fungi and bacteria. There are few fungi such as white rod fungi that are able to alter the lignin structure of the biomass by producing the enzyme called ligninase. This digesting enzyme degrades the lignin, by oxidizing the substituted aromatic compound of the lignin to aryl cation radicals (Zanirun, 2009), and thus exposing the internal part of cellulose to be accessed by cellulase. This technique has been proven as a promising lignocellulosic pretreatment method with mild conditions applied, no chemicals involved, low energy input and being environmental friendly (Sun and Cheng, 2002). However, the process is very slow and requires careful monitoring of growth condition in which makes the process complicated and unattractive commercially (Chandra *et al.*, 2007). Hamisan *et al.* (2009) did some of the comparative studies conducted to show the potential of biological pretreatment over chemical pretreatment.

Besides, the growth of fungi attacking and altering the OPEFB structure has also been done to demonstrate how the microorganism acts on lignocellulosic biomass as shown in Figure 9. Dense mycelia of *Botryosphaeria rhodina* UPM3 can be observed on the OPEFB fibre on 5th day of fermentation (Figure 9c). From viewing under SEM, the mycelia of *B. rhodina* UPM3 was found to be attached to OPEFB mainly on its pores and craters. Several observations have shown that *Botryosphaeriaceae* fungi infect the host plant via lenticels, stomata or openings. Therefore, this finding indicates that craters on the OPEFB surface play a vital role for fungal attachment. The mycelia network of *B. rhodina* UPM3 was of relatively high mass on the final day (7th day) of SSF and covered up all the area of OPEFB (Figure 9d). Indeed, SSF resembles the natural environment of fungus cultivation and thus making the resultant mycelia morphology more favourable for enzyme formation (Bahrin *et al.,* 2012b; Bahrin *et al.,* 2011).

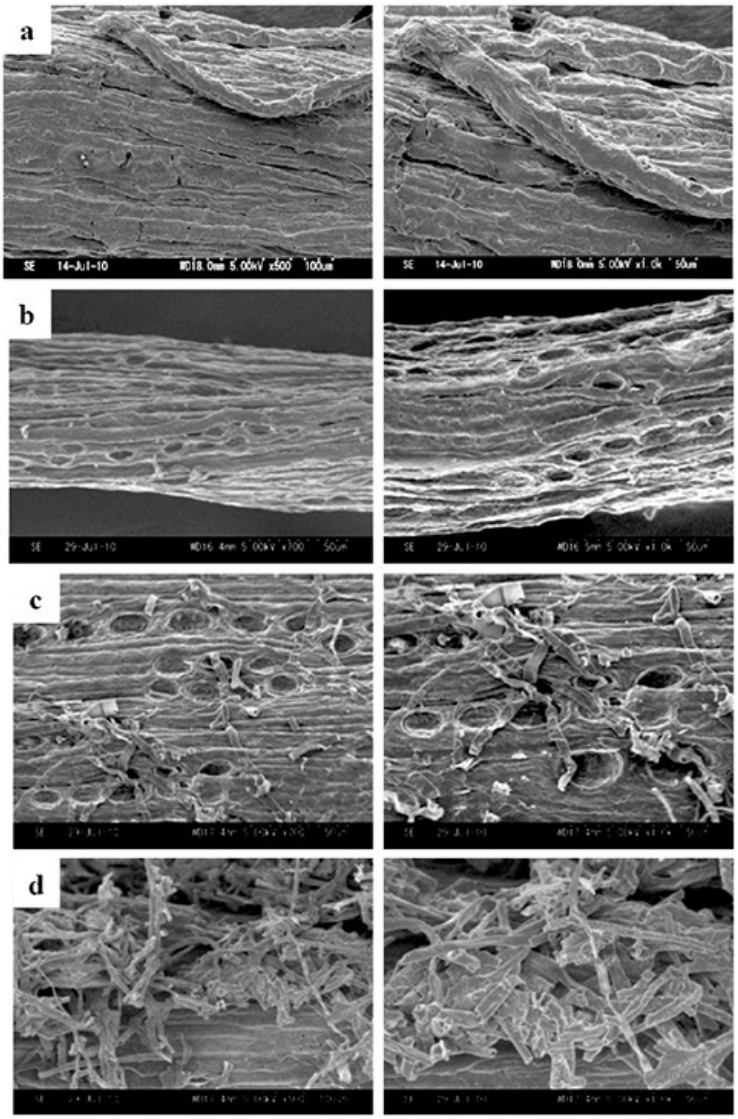

Figure 9 Scanning electron micrograph of OPEFB fibre fermented with *B. rhodina* UPM3 for (a) 0, (b) 3rd, (c) 5th and (d) 7th day of SSF (Source: Bahrin *et al.,* 2011)

Search on efficient pretreatment methods for lignocellulosic biomas is extensively being done in our research activities. Current data are as shown in Table 1. Among all the pretreatment processes, chemical pretreatment shows the best lignin degradation capability, with fast and low energy consumption, which is more efficient compared to other pretreatment methods. However, chemical pretreatment is not considered to be implemented in the production line since it is not environmental friendly. The acid or alkaline used in the process needs to be neutralized before being discharged to the environment. Therefore, improvement on the physical and biological pretreatments has become a new focus for the pretreatment of lignocellulosic biomass.

Table 1 Chemical compositions of various lignocellulosic biomass for cellulase and biosugars production

Lignocellulosic biomass	Pretreatment	Chemical composition (%)			Total potential sugars[a] (%)	References
		Cellulose	Hemicellulose	Lignin		
Oil palm decanter cake	Untreated	22	4	31	26	Razak *et al.* (2012)
	Pretreated with 1% NaOH with autoclaved at 121°C for 20 min	52[b]		16	52	
Oil palm empty fruit bunch	Untreated	39	21	19	50	Ibrahim *et al.* (2013) Bahrin *et al.* (2012a) Abu Bakar *et al.* (2010)
	Pretreated with 2% NaOH with autoclaved	60	22	12	82	
	Physical pretreatment using superheated steam (180°C, 60min)	46	27	19	73	Bahrin *et al.* (2011)
Sago pith residue	Untreated	26	15	7.5	41	Linggang *et al.* (2012) Jenol *et al.* (2014)
	Pretreated using Dextrozyme	44	15	5	59	
Rice straw	Untreated	25	15	23	40	Roslan *et al.* (2009)
	Chemical pretreatment using 0.5% NaOH with autoclaved	58	4	9	62	

[a]Total sugars is the total percentage of cellulose and hemicellulose
[b]Reported as total potential biosugars

A rough classification of the pretreatment methods can also be made according to the following (Galbe and Zacchi, 2007):

i. Acid-based methods, i.e. pretreatment at low pH, resulting in hydrolysis of the hemicellulose to monomer sugars and hence minimizing the need for hemicellulases.

ii. Methods working close to neutral conditions, e.g. steam pretreatment and hydrothermolysis, causing solubilization of most of the hemicellulose due to the acids released from the hemicellulose, e.g. acetic acid, while not usually resulting in total conversion to monomer sugars. This thus requires hemicellulases acting on soluble oligomer fractions of the hemicelluloses.

iii. Alkaline methods, that leaves a part of the hemicellulose, or in the case of ammonia fibre explosion, almost all hemicellulose in the solid fraction. This then requires hemicellulases acting both on solid and on dissolved hemicellulose. An alternative is to perform acid hydrolysis on this fraction, for instance after removal of the cellulose by enzymatic hydrolysis. This affects, of course, not only the method that should be used for assessment of the pretreatment but also the cost of the overall hydrolysis of the carbohydrates.

DEVELOPMENT OF ENZYME COCKTAIL SYSTEMS FOR BIOSUGARS PRODUCTION

The enzymes involved in degradation of lignocellulosic materials are very complex due to the complex structure of the lignocellulosic biomass itself. Since lignocellulosic biomass consists of lignin, hemicellulose and cellulose as its major components, full complex enzymes system is required to digest those components into simple sugars. Lignin requires a set of ligninolytic enzyme while hemicellulose requires a set of cellulolytic enzyme. Ligninolytic consisted of three major enzymes (i.e.: lignin peroxidase, manganese peroxidase and laccase) as mentioned by Zanirun (2009) while cellulolytic enzyme consists of endoglucanase, exoglucanase and βglucosidase (Ibrahim, 2013, Ibrahim *et al.,* 2012). However, some other enzymes like mannanase and xylase are still involved in degrading the lignocellulosic biomass (Abd-Aziz *et al.,* 2001, Ab. Razak, 2006). The enzyme produced by *Aspergillus niger* from palm kernel cake (PKC) has been profiled by Ong *et al.* (2004) and Gan (2005) which characterized mannanase as one of the enzymes produced in the system. The production has been statistically optimised (Abd-Aziz *et al.*, 2008) and extended using mixture of microorganisms, which are *A. niger* and *Sclerotium rolfsii* (Abd-Aziz *et al.*, 2009). Since mannase is only involved in converting mannose into glucose, detailed study on the enzyme system for complete lignocellulosic biomass degradation has been continued.

Ligninolytic Enzymes

The widely organisms to degrade lignin are the wood rotting fungi (Zanirun, 2009). They can be divided into three groups according to the morphology of the decay they caused in wood. White rot fungi is the most prominent fungi having the ability to

degrade wood in nature. Their approach is to delignify or modify the lignin structure so that the enzyme can access the cellulose and hemicellulose embedded within the lignin matrix. Lignin is an insoluble, high molecular weight polymer, so the initial steps in its biodegradation by white rot fungi must be extracellular. The presence of three major extracellular enzymes namely lignin peroxidase, manganese peroxidase and laccase are significantly involved in lignin degradation processes (Lovitt *et al.,* 1996; Ang, 2007). They are non-specific enzymes capable of degrading natural aromatic polymers of lignin. Generally, there are three major challenges faced by the white rot fungi in association with the facts that:

i. Lignin polymer is large, hence the ligninolytic system must be secreted extracellularly,

ii. Lignin structure is comprised of inter-unit C-C and ether bond C-O-C, therefore the degradation mechanism must be oxidative rather than hydrolytic, and

iii. Lignin polymer is stereo-irregular.

An intensive search for the extracellular enzymes associated with lignin degradation led to the discovery of extracellular peroxidases in *Phanerocahete chrysosporium* (Glenn *et al.*, 1983). Now it is thought that the ligninolytic system of *P. chrysosporium* consists of a pool of enzymes, namely lignin peroxidase, manganese peroxidase and H_2O_2-producing enzymes. Another lignin degrading enzyme, laccase, is not produced by *P. chrysosporium*, but it is formed by many other white rot fungi (Zanirun, 2009). Lignin degrading enzymes are also produced by bacteria as reported by (Rahman *et al.*, 2013).

Lignin Peroxidase (LiP)

In 1983, two groups announced the discovery of an extracellular H_2O_2-requiring enzyme activity that catalyzed several of the reactions formerly obtained with intact cultures of *P. chrysosporium* (Glenn *et al.*, 1983). Lignin peroxidase (once called ligninase) (EC 1.11.14) has been purified and characterized (Tien and Kirk, 1984). The enzyme is a glycoprotein that contains about 15% carbohydrates and an iron protoporphyrin IX (heme) as a prosthetic group. It has a molecular weight of 41000-42000 Da and a pH optimum of 2, but the enzyme is unstable at this low pH. The lignin peroxidase family contains multiple isoenzymes. The number of isoenzymes reflects differences in strains, culture conditions and purification/ fractionation techniques (Kirk and Farrell, 1987). The enzyme can be assayed by the oxidation of veratryl alcohol to veratraldehyde, the formation of which is monitored at 310 nm (Tien and Kirk, 1984). Lignin peroxidase has no substrate specificity, reacting with a wide variety of lignin model compounds and related compounds (Zanirun, 2009). Lignin peroxidase can oxidize both phenolic and non-phenolic lignin related compounds resulting in cleavage of the C_α - C_β bond, the aryl C_α bond, aromatic ring opening, phenolic oxidation and demethoxylation.

Manganese Peroxidase (MnP)

Manganese peroxidase (EC 1.11.13) is also a he me peroxidase and it forms a family of isoenzymes. Similarly to LiP they are also glycoproteins, with one iron protoporphyrin IX group per mol of enzyme. The molecular weight is approximately 46000 (Glenn and Gold, 1985). The MnP can oxidize a variety of phenols and dyes (Kuwahara *et al.*, 1984). The catalytic cycle of MnP is esentially the same as for LiP with the exception that Mn(II) is necessary to complete the cycle.

Laccase

Laccase (EC 1.10.3.2) is a copper-containing polyphenol oxidase enzyme which reduces oxygen by the oxidation of a phenolic substrate. Substrate oxidation by laccase is a one-electron reaction generating a free radical (Ang, 2007). As LiP and MnP, laccase gives both polymerization and depolymerization of lignin (Bourbonnais and Paice, 1990). It has been shown that the artificial laccase substrate, ABTS has the capacity to act as a mediator, enabling the oxidation of non-phenolic lignin model compounds that are not laccase substrates on their own (Bourbonnais and Paice, 1990). Bleaching of lignin-containing pulps can also be achieved with laccase and by the use of low-molecular weight redox-mediators (such as hydroxybenzotriazole) (Call and Mücke, 1995). According to recent results, a laccase with a molecular weight of 46500 Da was found in *P. chrysosporium*. The molecular weight is the same as that of MnP and, unless it contains copper in the structure, it could probably be a form of MnP (Zanirun, 2009).

Enhancement of Ligninolytic Enzymes Activities

In essence, the important characteristics of extracellular, oxidative and unspecific enzymes catalyse initial depolymerization of lignin. Mediators are the actual oxidants responsible for lignin degradation and can penetrate deeply into the lignocellulosic matrix due to their small size. They are defined as low-molecular-weight substrates that facilitate enzymatic oxidation by generating stable high-potential intermediates which are later involved in chemical (non-enzymatic) reactions with other compounds, following diffusion-controlled kinetics. Therefore, an approach of biological treatment on OPEFB for partial removal or modification of its lignin

structures has been on going over the past years which started with the isolation and screening of microorganisms in our laboratory, followed by optimization of ligninolytic and enzyme productions. Previously, Zanirun *et al.* (2009) conducted the optimization of lignin peroxidase (LiP) enzyme from *Pycnoporus* sp. using 2-level factorial design and the results showed that 51 U/L of LiP was successfully achieved at 24mM nitrogen concentration, pH 3.5, agitation speed at 110 rpm and veratryl alcohol (1 mM) as inducers. In the meantime, an idea of using crude ligninolytic enzyme produced from locally isolated white rot fungi namely *P. sanguineus* UPM4 as a preferred pretreatment method to divert the usage of chemical is now becoming our major intention. The performances of the collected crude enzymes were compared with the addition of synthetic mediator of HBT and the result of cellulose hydrolysis is as shown in Figure 10 (Unpublished data). Up to 30 g/L of sugar was produced with HBT addition compared to application of crude ligninolytic enzymes alone. The addition of mediator to the ligninolytic crudes played a big role in enhancing the enzymatic hydrolysis of cellulose as the mediators will increase the binding sites of the ligninolytic enzymes to lignin surfaces and therefore modifying the structure and partial removal. Our current mission to develop green biological approaches with milder processes could benefit the environment against chemical treatment. The potential of ligninolytic enzymes as biological treatment to enhance enzymatic hydrolysis of cellulose could be enhanced further since there is room for improvement.

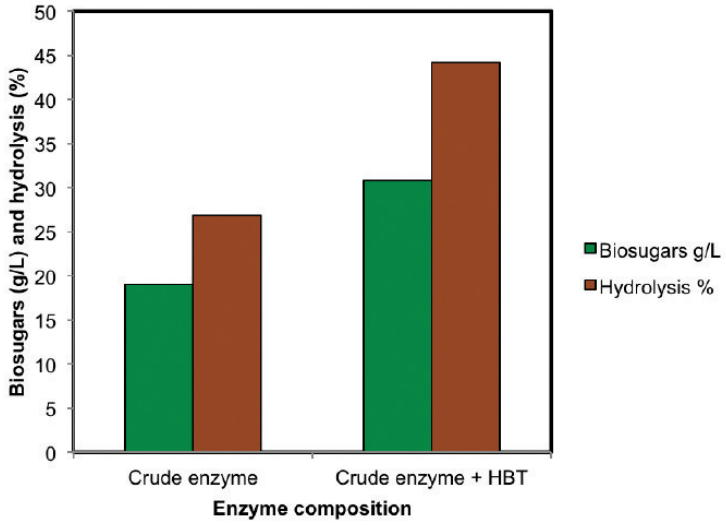

Figure 10 The effect of HBT as mediator on the enzymatic hydrolysis of OPEFB (Unpublished data)

Cellulolytic Enzymes

Cellulase, also known as cellulolytic enzyme refers to a class of enzymes produced by fungi, bacteria and protozoa that is able to hydrolyze the polymer structure of cellulosic materials into its fragments or monomers. Cellulase consists of three types of enzymes i.e.: endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) that work synergistically to degrade the complex cellulosic structure into its monomers (Mathew *et al.*, 2008). The mechanism of cellulose degradation by cellulase is shown in Figure 11. The endoglucanase or 1,4-β-D-glucangluconohydrolyase acts on carboxy methyl cellulose (the crystalline region of lignocellulosic biomass) that randomly cleaves the cellulose chain yielding cello-oligosaccharides and few amounts of glucose monomers. The exoglucanase or 1,4-β-D-

glucancellobiohydrolase acts on the microcrystalline section of non-reducing end of cellulose producing two or three molecules structure of glucose known as cellobiose. The cellobiose is further simplified by 1,4-β-glucosidase that facilitates the hydrolysis of cellobiose to glucose monomers. These three enzymes target the specific cleavage of β-1,4-glycosidic bond (Wood and McCrae, 1979).

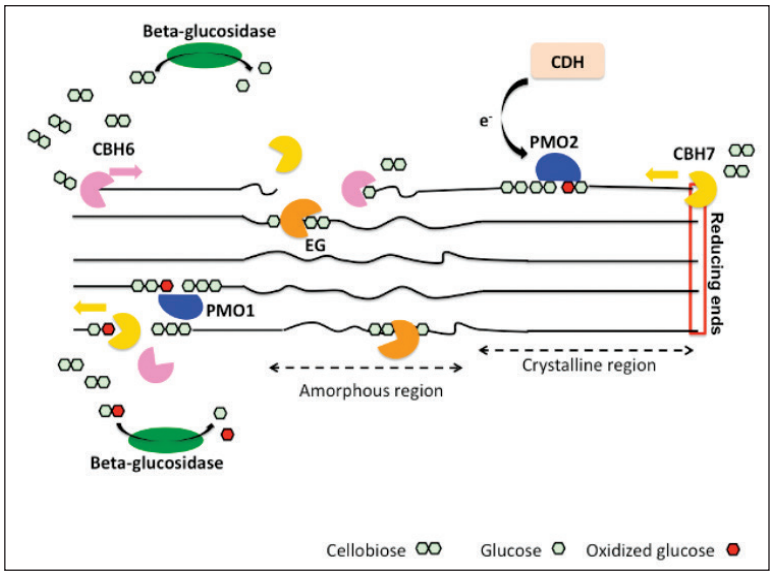

Figure 11 A simplified scheme of the current view on the enzymatic degradation of cellulose, involving cellobiohydrolases (CBH), endoglucanases (EG), type I and type II PMOs (PMO1 and PMO2, respectively). Cellobiose dehydrogenase (CDH) is a potential electron donor for PMOs. EGs and PMOs cleave cellulose chains internally releasing chain ends that are targeted by CBHs. CBHs generate cellobiose or oxidized cellobiose that are subsequently hydrolyzed by by β-glucosidase

(Source: Dimarogona *et al.,* 2012)

Exoglucanase

Exo-1,4-β-D-glucanase is also recognized as cellobiohydrolase, exocellulase, Avicelase or FPase. Avicelase and FPase are named according to the substrates used in the enzyme assay to measure the activity (Wood and Bhat, 1988). Exoglucanase is very crucial for hydrolyzing microcrystalline cellulose and very specific in cleaving β-1,4 linkages of the cellulose chain. However, these enzymes are inactive on cellobiose and substitute celluloses. Both endoglucanases and exoglucanases are active on amorphous region of cellulose. However, only exoglucanase can degrade crystalline region of cellulose chain.

The class I enzymes CBH I (from *Trichoderma reesei* and two endoglucanases E4 and E6 from *Thermobifida fusca*) prefer to hydrolyse the cellulose polymer from the reducing end, whereas class II or CBH II (CBH II from *T. reesei* and E3 from *T. fusca*) liberate cellobiose from the non-reducing end (Barr *et al.*, 1996). In *T. reesei* cellulase system, CBH I and CBH II are the major components from the total cellulase protein, constituting 60% and 20%, respectively. The principal product of CBH I and CHB II activity is cellobiose which consequently inhibits the activity of cellobiohydrolases and endoglucanases.

Endoglucanase

Endoglucanases react very particularly to break down the internal β-1,4 glycosidic from amorphous, swollen, substituted celluloses (carboxymethyl and hydroxylmethyl cellulose) and cello-oligosaccharides. Endo-1,4-β-D-glucanase is also known as endoglucanase, endocellulase and CMCase (Wood and Bhat, 1988). The most applied enzyme assay to analyse this enzyme

uses carboxymethyl cellulose as a substrate and thus this enzyme is described as CMCase. The specificity of endoglucanases towards crystalline cellulose and cellobiose is very poor. Bhat *et al.* (1993) reported remarkable differences between endoglucanases activities by *Penicillium pinophilum* when utilizing substituted, unsubstituted and reduced cello-oligosaccharides as substrates.

Multiple endoglucanases are produced by bacteria and fungi with a broad range of substrate specificity to hydrolyse cellulosic material efficiently. *T. reesei* is capable of secreting five types of endoglucanases, designated as EGI, EGII, EGIII, EGIV, and EGV. Endoglucanases III and IV were only active on cellotriose and higher cello-oligosaccharides, while endoglucanases II and V required 4-6 glucose residues to be activated (Lynd *et al.*, 2002).

β-Glucosidase

β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose. These enzymes have been recognized as the enzymes that prevent cellobiose accumulation and cellulase inhibition. β-Glucosidase enzyme can be categorized as either aryl β-D-glucosidases (hydrolyzing exclusively aryl-β-D-glycosides), cellobiases (hydrolyzing diglucosides and cello-oligosaccharides) or β-glucosidases with broad substrate specificities. Most β-glucosidases exhibit wide substrate specificities and hydrolyse aryl, alkyl β-D-glycosides, β-1,1-, β-1,2-, β-1,3-, β-1,4- and β-1,6-linked diglucosides, as well as substituted and unsubstituted cello-oligosaccharides. An attractive finding showed that intracellular β-glucosidase from *S. thermophile* was found to be an aryl-β-glucosidase, whereas two extracellular β-glucosidases from the same organism hydrolyzed only cellobiose (Bhat *et al.*, 1993).

Cellulolytic Enzyme Cocktail System

The search of suitable cellulase for lignocellulosic material degradation is extensively carried out in our research activities. Numerous microorganisms have been isolated and characterized to find the best cellulase producer. Thermophilic cellulase producing bacteria has been isolated from compost made of OPEFB and POME sludge. This thermophilic bacteria is aimed to degrade lignocellulosic materials at high temperature (Baharuddin *et al.,* 2010). A locally isolated fungus *A. niger* EB1 has been employed to produce crude cellulase from OPEFB (Noratiqah *et al.,* 2013). Mannanase production by *A. niger* FTCC 5003 from oil palm kernel cake has also been carried out (Ong, 2006). Two strains (*T. asperellum* UPM1 and *A. fumigatus* UPM2) have been isolated from OPEFB by Abu Bakar *et al.* (2010). Those isolated microorganisms were tested on different types of lignocellulosic biomass using submerge (SmF) and/or solid-state fermentation (SSF). For example, the production of cellulase from by *A. fumigatus* SK1 through SSF has been done by Ang *et al.* (2013). Various types of substrates have and currently being tested for cellulase production as well as for enzymatic hydrolysis, *i.e.*: POME solid (Wong, 2005), oil palm kernel cake (Ong, 2006), SPR (Linggang *et al.,* 2012), OPDC (Razak *et al.,* 2012), OPEFB (Bahrin *et al.,* 2011, Abu Bakar *et al.,* 2010, Ibrahim *et al.,* 2013, Zanirun *et al.*, 2014) and OPT (Ang *et al.,* 2013). Difficulties occurred since different microorganisms will act differently on different substrates to produce cellulase. Out of many techniques, substrates and strains that have been tested, two fungi strains (*T. asperellum* UPM1 and *A. fumigatus* UPM2) were found to produce significantly higher cellulase activities compared to others. Therefore, extensive research has now being conducted to explore the capabilities of these two strains for cellulase production.

Fungi *T. asperellum* UPM1 and *A. fumigatus* UPM2 have been deposited for patent filing at DSMZ with code numbers of DSMZ 24606 and DSMZ 24607, respectively. In our findings, *T. asperellum* UPM1 produced higher β-glucosidase than FPase and CMCase while *A. fumigatus* UPM2 produced higher CMCase and FPase than β-glucosidase. The importance of the interaction between cellulase components (CMCase, FPase and β-glucosidase) had been studied by Ibrahim (2013). Variation of cellulase activities produced by two different fungi strains has lead a research on developing crude cellulase cocktail that can improved cellulose degradation. The combination of cellulase from *T. asperellum* UPM1 and *A. fumigatus* UPM2 has produced better cellulase cocktail system compared to cellulase from one strain. The process to produce this crude cellulase cocktail and its composition has been patented (Abd-Aziz *et al.,* 2011). The performance of this patented crude cellulase cocktail was comparable with the commercial purified cellulase available in the market (Celluclast 1.5 L produced by Novozyme, Denmark). The comparison was based on the specific enzyme activity and the amount of biosugars produced after hydrolysing the pretreated OPEFB, with data as shown in Table 2.

Table 2 Specific crude cellulases activities and hydrolysis performance of crude cellulases cocktail in comparison with Celluclast
(Source: Ibrahim, 2013)

Cellulase	Specific cellulase activity (U/mg)			Bio sugars (g/L)	Hydrolysis percentage (%)	Hydrolysis yield (g/g)
	β–glucosidase[a]	CMCase[b]	FPase[b]			
Crude cellulases cocktail[c]	7.23	89.17	3.08	29.7	73	0.59
Celluclast	1.44	95.88	4.20	31.1	76	0.64

[a]Cellulase produced at initial pH 7.0 and at temperature 35°C
[b]Cellulase produced at initial pH 6.0 and at temperature 45°C
[c]Cellulase prepared at the ratio of 3:2 of UPM1:UPM2

The rate of enzymatic hydrolysis on cellulosic material by cellulase is determined by the amount of enzyme adsorbed on cellulose surface area. Thus, many studies on adsorption kinetic have been explored to estimate the cellulase action on cellulose. However, those models including Michaelis-Menten kinetics are not able to specifically determine the exact hydrolysis model of cellulase. This is because the structural features such as surface area, crystallinity and the presence of other substances like lignin differ from one kind of lignocellulosic biomass to another. Inhibition of the cellulase action by reaction products was also reported which lead to the multiplicity and complexity of the cellulase mechanism (Ibrahim, 2013). Reese (1977) concluded that enzyme adsorption on cellulose is dependent on concentration of enzyme, nature and the amount of substrate available, surface area, physical properties of the enzyme and hydrolysis environment. Yang *et al.* (2011) mentioned that the cellulase action is also dependent on enzyme-related factors like enzyme source, product inhibition, thermal inactivation, activity balance for synergism, specific activity, nonspecific binding, enzyme processibility and enzyme compatibility. However, according to our findings (Ibrahim *et al.,* 2013, Linggang *et al.,* 2012, Razak *et al.,* 2012, and Abu Bakar *et al.,* 2012), the cellulases work differently when different substrates were used. This is due to the complexity of the cellulose structure as well as the complexity of the cellulase system.

The rate of adsorption is proportional to the amount of cellulase in the system. Increase in enzyme concentration will increase the number of cellulase that bind to the cellulose structure and allow for rapid degradation (Walker and Wilson, 1991). However, a concrete conclusion on the nature of the substrate's structural features is difficult to be determined due to the variation of the lignocellulosic structure. Moreover, different pretreatments on

the lignocellulosic biomass further contribute towards variation of structural features and thus varying the hydrolysis performance obtained (Bahrin, 2012). During hydrolysis, the cellulosic material undergoes fragmentation which increases the surface area and changes the crystallinity percentage of the substrate (Bahrin *et al.*, 2012b), which differ according to the type of cellulosic material and complexity of the cellulase system. Besides, the soluble matter released during the pretreatment and/or during hydrolysis has been suggested as one of the inhibitory compounds towards the action of cellulase. Those compounds include the chemicals being used during pretreatment, and the phenolic and aromatic compounds released during the degradation (Razak *et al.,* 2012; Linggang *et al.,* 2012), which differ based on the type of lignocellulosic biomass. As synergistic effects between cellulases are influenced by the nature of the substrate, such as chemical composition, it is often challenging to compare research results in literature using different substrates.

In terms of physical properties of the cellulase, the endoglucanase and the exoglucanase (so called cellobiohydrolyase - CBH) have a catalytic domain (CD) and cellulose-binding domain (CBD). The CBD functions to make sure the enzyme interact with cellulose at the correct orientation while CD is connected with CBD to help them bind with the cellulose (Binod *et al.*, 2011). Removing one of these domains from the cellulase will impair the hydrolysis action. The cellulase domain can also be interfered by the presence of inhibitory compounds like furfural, acids and phenolic substances. Endoglucanase and CBH account for most of the cellulase activity. However, a sufficient amount of β-glucosidase is needed in order to reduce the inhibitory effect of cellobiose to endoglucanase and CBH (Walker and Wilson, 1991). A synergistic enzyme effect between the cellulase components was revised by Yang *et al.* (2011). Since cellulase components are different when employing different

microorganisms, such synergism effect is dependent on cellulase source and even substrate feature. For example, the interaction between the CD and CBD was observed on cotton fibre but was not on microcrystalline cellulose (Din *et al.*, 1995). In addition, although it was found that β-glucosidase is a rate-limiting enzyme in hydrolysis (Ibrahim *et al.*, 2013, Linggang *et al.*, 2012), a sufficient amount of endoglucanase and CBH are also important. Duff and Muray (1996) reported that a ratio of β-glucosidase to CBH in the range of 0.12-1.5 gave the best hydrolysis performance. However, recent study suggested that the optimal enzyme ratios are also affected by the type and source of cellulosic material and the pretreatment that had been applied on it (Yang *et al.*, 2011).

The hydrolysis performances by crude cellulase produced by *T. asperellum* UPM1 and *A. fumigatus* UPM2 act on different types of local lignocellulosic biomass are shown in Table 3. Although all the substrates were pretreated using the same method (using 2% NaOH with autoclaved), however, each of them required different amount of cellulase activities.

Lignocellulolytic Enzyme Cocktail System

The crude lignocellulolytic enzyme cocktail is a mixture of crude ligninase enzyme extract and crude cellulase cocktail synthesized from *T. asperellum* UPM1 and *A. fumigatus* UPM2 (Ibrahim *et al.,* 2013 and Unpublished data). Enzymes involved in lignin degradation are commonly referred as ligninase, while cellulase refers to enzymes responsible for cellulose hydrolysis. Ligninase will act on lignin to increase cellulase accessibility towards cellulose. Then, cellulase will further breakdown cellulose into simpler sugar monomers. Lignin is a complex structure made up of heteropolymer consisting of three phenyl propionic alcohols monomers which are coniferyl alcohol, coumaryl alcohol, and

sinapyl alcohol (Kumar *et al.,* 2009). Common ligninase types involved in lignin degradation are phenol oxidases, peroxidases, and dehydrogenases. Phenol oxidase works by oxidizing phenolic parts in lignin using oxygen as an electron acceptor. The most studied and dominant phenol oxidase is laccase, a multicopper enzyme which falls into phenol oxidase enzyme category as it attacks phenolic parts in lignin. Meanwhile, peroxidases have Fe-containing haem prosthetic group that uses H_2O_2 as an electron acceptor during redox process. Among the three phenyl propionic alcohol units of lignin, coniferyl alcohol is more favourable to be oxidized by peroxidases compared to sinapyl alcohol (Ralph *et al.,* 2004). In addition, dehydrogenases transfer hydride groups from a substrate to an acceptor such as NAD^+ (Burns *et al.,* 2013). The advantage of using crude lignocellulolytic enzyme cocktail is the treatment time is expected to be shorter and hence making the biological treatment process simpler in one vessel within a single step or one go simultaneously, in turn making the process of biosugar production easier.

CONVERSION OF PRETREATED LIGNOCELLULOSIC BIOMASS TO BIOSUGARS

Conversion of pretreated lignocellulosic biomass into biosugars can be done by three different methods, which are chemical, physical and biological processes. Many studies have been intensively conducted to find the most effective method to produce higher yield of biosugars, while at the same time being environmental friendly. Biological method is the most promising method, as the process employs microorganisms and/or enzymes to degrade the biomass and produce biosugars.

In the hydrolysis of cellulosic component in lignocellulosic biomass, cellulase, which contains exoglucanase, endoglucanase and β-glucosidase are responsible in converting carbohydrate into biosugars. In the biosugar hydrolysate, there are several types of sugars, which are glucose, arabinose, rhamnose, maltose and xylose (Razak *et al.*, 2012). The composition of each biosugar differs according to biomass. However, in general, glucose was found to be the most abundant hexose in the hydrolysate. This happens due to the major components of lignocellulosic biomass is cellulose.

A study conducted by Ibrahim *et al.* (2013) has reported that 32.2 g/L biosugars was recovered (73.2% of hydrolysis percentage) from OPEFB using 5% (v/v) enzyme loading, while 4.37 g/L polyoses (biosugars) has been obtained from OPDC with 69.4% hydrolysis percentage (Razak *et al.,* 2013). Different results obtained might be due to the amount of lignin present in the biomass. As for the OPEFB and OPDC, the lignin contents were 12.3% and 16.6%, respectively (Ibrahim *et al.,* 2013; Razak *et al.,* 2013), while SPR is 4.9% (Linggang *et al*., 2012). On the other hand, this could be reflective of the cellulosic materials contained in the biomass, since OPEFB has higher potential sugars as compared to OPDC. Although fine structure like dried POME was used, the biosugars obtained were not as higher as those in OPEFB (Wong *et al.*, 2008). Roslan *et al.* (2011) has produced the highest glucose concentration (0.38 g/g rice straw) by using 20 cycle disc milled with thermal of sample. However, the aforementioned authors claimed that, the value of hydrolysis percentage was slightly higher than cellulose percentage present in the rice straw. Thus, it was suggested that glucose recovered originated from the degradation of hemicellulose.

Table 3 Overview for hydrolysis products of several pretreated lignocellulosic biomass

Biomass	Pretreatment	Saccharification	Biosugars obtained (g/L)	Hydrolysis percentage (%)	Hydrolysis yield (g/g)	References
Oil palm empty fruit bunch	Chemical pretreatment using 2% NaOH with autoclaved	Crude cellulases cocktail of UPM1:UPM2 (Ratio 3:2)	32.2	73	0.59	Ibrahim *et al.* (2013)
Oil palm decanter cake	Chemical pretreatment using 1% NaOH with autoclaved	Crude cellulases cocktail of UPM1:UPM2 (Ratio 1:4)	4.4	69	0.35	Razak *et al.* (2012)
Sago pith residue	Biological pretreatment using Dextrozyme	Crude cellulase of UPM2	20.8	73	0.42	Linggang *et al.* (2012)
Sago pith residue	Biological pretreatment using Dextrozyme	Crude cellulase of UPM2	20.7	71	0.40	Jenol *et al.* (2014)
Rice straw	Mechanical pretreatment using Disc mill	**Aspergillus* sp.	-	77	0.38	Roslan *et al.* (2011)

UPM1 is *T. asperellum* and UPM2 is *A. fumigatus*

*Cellulase produced via SSF

BIOCONVERSION OF BIOSUGARS TO BIOFUEL

For the past few years, the world has been facing the problems of global warming and climate change, instability petrol prices, depletion of petroleum reservoir as well as serious environmental pollution due to consumption of fossil fuel for generating energy (Garcia *et al.*, 2011). This has happened due to the increase in the number of human population, which subsequently contributes to higher demand in energy for industrial activities, transportations and households' energy consumption. Utilisation of fossil fuel has been reported as the major contributor to the increase of carbon dioxide percentage in the atmosphere (Florides and Christodoulides, 2009). This phenomenon has contributed to the increase of world temperature that causes several of the worlds' environmental problems (Intergovernmental Panel on Climate Change, 2007). Thus, the challenge to develop alternative energy that is renewable, clean and environmental friendly has been tackled by many researchers in order to shape the future of energy technology. The importance and overview of biofuel production in Malaysia have been described by Abd-Aziz and Ibrahim (2014). To this aim, biomass can generate a variety of value added products, including biofuel, which can serve as an alternative energy supply for the world. The recent interest in this biorefinery concept is based on the mitigation of climate change by substituting the biomass energy for petroleum or other fossil-fuel energy (Abd-Aziz *et al.,* 2013). Currently, bioethanol, biobutanol and biohydrogen are examples of biofuels that offer potential based on the following present research data.

Bioethanol

Bioethanol produced from renewable biomass is attracting global attention as an alternative energy source. Lignocellulosic biomass is the most promising feedstock considering its great availability and low cost. Due to these facts, more studies and collaborations are required to improve biotechnological production of bioethanol from lignocellulosic biomass with research being done from the upstream level to downstream. On our part, a recombinant yeast inserted with glucoamylase and α-amylase was developed to produce bioethanol from gelatinised sago starch (Abd-Aziz *et al.,* 2001; Ang *et al.,* 2001) and the characteristics of theses enzymes have been determined to explain their mechanism in hydrolysis of starch to glucose for bioethanol production (Ang *et al.,* 2001). It has first been developed to produce biosugars from gelatinised sago starch (Nazri, 2004) before those biosugars are subjected for bioethanol production through direct fermentation (Ang, 2001, Ang et al., 2002), with further study on the effect of carbon to nitrogen ratio (Abd-Aziz *et al.,* 2001).

One of the most challenging factors in second generation of bioethanol from lignocellulosic biomass is the high cost of cellulase enzymes. Nevertheless, crude cellulolytic enzymes produced from lignocellulosic feedstock help to reduce the cost of enzyme production significantly. OPEFB was used as a substrate in the production of cellulase by locally isolated fungi, *T. asperellum* UPM1 and *A. fumigatus* UPM2 in submerged fermentation (Abu Bakar, 2011; Abd-Aziz *et al.,* 2008). Crude cellulase cocktails from both fungi produced 8.37 g/L of biosugars with 0.17 g/g yield during saccharification process. The biosugars from OPEFB hydrolysate were successfully fermented into bioethanol by Baker's yeast with 0.59 g/L ethanol, corresponding to 13.8% of the theoretical yield (Abu Bakar *et al.,* 2012) as shown in Table 4. Besides, bioethanol

production has also been produced from biosugars obtained from hydrolysis of OPEFB in SSF as reported by Bahrin (2012). Optimization of bioethanol production has been conducted by Roslan (2011) using rice straw as substrate.

Sago *hampas* consists of lignocellulosic component and of additional interest, this biomass also has residual starch fraction that can be recovered. On dry basis, sago *hampas* contains 58% starch, 23% cellulose, 9.2% hemicellulose, and 4% lignin (Linggang *et al.,* 2012). In one attempt by Awg-Adeni *et al.* (2013), the starch from sago *hampas* was hydrolysed enzymatically for three cycles. However, to enhance concentration of glucose from 7% substrate load of sago *hampas* is currently not possible. Thus, an alternative method termed as cycles I, II, and III which involves reusing the hydrolysate for subsequent enzymatic hydrolysis process was introduced. Greater improvement of glucose concentration (138.45 g/L) and better conversion yield (52.72%) were achieved with the completion of three cycles of hydrolysis. In comparison, cycle I and cycle II had glucose concentration of 27.79 g/L and 73.00 g/L, respectively. The glucose obtained was subsequently tested as substrate for bioethanol production using commercial Baker's yeast. The fermentation process produced 40.30 g/L of ethanol after16 h, which was equivalent to 93.29% of theoretical yield based on total glucose existing in fermentation media.

Table 4 Comparison data of bioethanol production from lignocellulosic biomass

Substrate	Hydrolysis method	Biosugars (g/L)	Ethanol (g/L)	Ethanol yield (g/g glucose)	Theoretical yield (%)	Reference
Commercial glucose	-	11.37	2.98	0.26	51	Abu Bakar *et al.* (2012)
OPEFB hydrolysate	Crude cellulase	8.37	0.59	0.18	14	Abu Bakar *et al.* (2012)
OPEFB hydrolysate	Commercial cellulase	20.0	9.4	0.47	10	Simarani *et al.* (2009)
OPEFB hydrolysate	Acid hydrolysis	32.0	14.76	0.46	87	Zainuddin *et al.* (2012)

Biobutanol

Butanol represents the next significant change required to meet the growth in demand for environmentally responsible and renewable fuel for transportation. Butanol ($C_4H_{10}O$) or butyl alcohol is an alcohol that can be used as a solvent or fuel, which can be produced by microorganisms through acetone-butanol-ethanol (ABE) fermentation (Ibrahim, 2013). Compared to other alcoholic fuels (ethanol and methanol), butanol has a higher heating value. Being a four carbon alcohol, it contains 25% more energy than ethanol which reduces fuel consumption and makes better mileage obtainable. It has lower volatility rate, less ignition problem and higher viscosity, properties which are almost similar to those of gasoline. Thus, it can be used in the existing engine system without any modifications (Jin *et al.*, 2011; Durre, 2007). Butanol can also be distributed through the current pipeline system because it is less corrosive compared to ethanol and methanol (Durre, 2007). These properties of butanol which as noted, makes it better than other alcoholic fuels and are almost similar to those of gasoline, thus making it a great renewable energy source if the production of butanol can be produced at lower cost.

Several approaches have been tested to produce biobutanol from lignocellulosic biomass by different strains of *Clostridium acetobutylicum* which including P262, NCIM 8502 (Madihah, 2004), and ATCC824 (Ibrahim, 2013; Razak, 2013; Linggang, 2013). Some local isolates like *C. butyricum* EB6 has also been tested for biobutanol production (Ibrahim *et al.*, 2012). In the early stages, biobutanol was produced from sago starch in the presence of partially purified a-amylase and glucoamylase via direct fermentation (Madihah *et al.,* 2001a) and its characteristics have been determined (Madihah *et al.*, 2000). In addition to this, the system was also used on gelatinised sago starch (Madihah *et*

al., 2001b), of which both are considered first generation biofuel. Now, production has been continued using lignocellulosic biomass instead as substrate. Three potential substrates such as OPEFB (Ibrahim, 2013), SPR (Linggang, 2013) and OPDC (Razak, 2013) have been used to produce biobutanol. All of these three types of lignocellulosic biomass have shown a comparable biobutanol yield, between 0.10-0.14 g/g of biosugars. Optimization on biobutanol production has been done using biosugars from OPDC with the biobutanol yield of 0.11 g/g and biobutanol concentration of 6.04 g/L (Razak *et al.*, 2013). Biobutanol production in 2-L bioreactor has also been tested using our local lignocellulosic biomass (Mohamad Remli, 2014). However, although many researches have been done, some improvements are still necessary in order to make this type of biofuel reliable in the industry. Some of the challenges include the acid inhibition phenomenon mentioned by Ibrahim *et al.* (2012), low biobutanol yield (Ibrahim *et al*., 2012), presents of toxic components (i.e.: furfural and phenolic compound) released from hydrolysis of lignocellulosic biomass which inhibit the cell growth (Razak *et al.*, 2013) and parameters variation (Linggang *et al.*, 2013).

Our current approach is through simultaneous saccharification and ABE fermentation. This approach is to reduce the number of steps involved in the production of biobutanol. A usual approach is by conducting saccharification and ABE fermentation separately which involves substrate pretreatment, saccharification, sugar recovery and fermentation. Each of the processes contributed to significant time consumption and cost for materials and apparatus. Any combination of these processes into a single step or step that can be carried out simultaneously may reduce the cost and time. The simultaneous process has been tested using pretreated OPEFB and sago pith residues (SPR) as substrate (Abd-Aziz *et al.*, 2014).

Both substrates have undergone fermentation via both separate and simultaneous processes by employing *C. acetobutylicum* ATCC 824 as inoculum. It showed that, the biobutanol yield obtained from simultaneous process is comparable to those found in separate process with the values of 0.14-0.19 g/g and 0.10-0.18 g/g for OPEFB and SPR, respectively (Abd-Aziz *et al.,* 2014). Our calculation showed that, the overall yield (from raw material until final biobutanol production) is better when using simultaneous process (0.09 g/g) instead of separate process (0.06 g/g), with reduction in total time consumed from 12 days for separate process to 8 days for simultaneous process (Ibrahim *et al.,* 2014). With these present data, biobutanol production has the potential to be our fuel for the future, provided improvements are made from time to time.

Biohydrogen

Besides bioethanol and biobutanol, biohydrogen production from biosugars has been reviewed as one of the most promising technologies in its field. Jenol (2014) has demonstrated the study on biohydrogen production of sago biomass (sago *hampas* and SPR) hydrolysate by *C. butyricum* A1 and *C. butyricum* EB6, both of which are local isolates. It was noted that 2.23 mol of H_2/mol of glucose has been yielded from the fermentation by using 10 g/L-glucose contained in SPR hydrolysate, which is higher than a well-known biohydrogen producer, *C. butyricum* EB6. Sago *hampas* has produced even higher yield which is 2.60 mol of H_2/mol of glucose. Previously, Chong *et al.* (2009) has reported that *C. butyricum* EB6 produced 3195 mL/L-medium of biohydrogen from POME, which was similar as those obtained by Jenol *et al.* (2014) using the same strain. This is higher than biohydrogen produced by *Ochrobactrum* sp. EB2 using POME as substrates (Chung, 2007). Yusoff *et al.* (2009) has also conducted biohydrogen production from POME

but by a mixed culture of natural inoculum instead. Statistically analysis has also been done to optimise the fermentation condition for biohydrogen production by natural microflora in POME (Rasdi *et al.*, 2009). The biohydrogen production has been done in 50-L bioreactor with the yield of 1130 NmL/L-POME. The advantage of using mixed culture of natural inoculum is that, the process can be done in non-sterile condition (Yusoff, 2010). Despite these production values, further findings are pivotal in development of biohydrogen as main form of bioenergy in future.

In addition to isolation of local biohydrogen producers and testing on different types of lignocellulosic substrates, research on co-production of biohydrogen and biobutanol has also been conducted. This research is still in its infancy where it has so far been tested only on pretreated OPEFB. The idea of producing biobutanol and biohydrogen in one system has begun with the understanding that *Clostridium* sp. produces these two biofuels through their metabolic pathways, without interfering each other (Ibrahim, 2013). This is because the biohydrogen is produced during the acidogenic stage while biobutanol is produced during the solventogenic stage. With the aim of reducing biofuel production costs, co-production has also been tested in simultaneous saccharification and ABE fermentation process. From this, 2366 mL/L medium of biohydrogen was produced from pretreated OPEFB in two-step process as compared to 2747 mL/L medium produced in simultaneous process (unpublished data). This value is also comparable to the biohydrogen production obtained by Jenol *et al.* (2014) and Chong *et al.* (2009), using SPR and POME substrates, respectively.

A WAY FORWARD

The discovery of new enzymes in bacteria, fungi and yeast will enable the conversion of non-food plant biomass namely lignocellulosic biomass into simple sugars namely biosugars that can be subsequently fermented to produce biofuel. For sustainable production to be achieved, there is a need to optimise the energy output in order to minimize the negative impact on the environment, socially and economically. There is also a need to optimise the release of sugars from plant cell walls in agricultural and wood-industry wastes to produce fermentable feedstock that microorganisms can convert to biofuel. Developing robust microbial strains that can use these feedstocks will enable sustainable production of biofuel.

With the use of enzymes, the breakdown of lignocellulosic biomass to release biosugars for fermentation becomes easier. In plants, the sugars are locked into the cell walls as long chain polymers in ways which currently do not fully understand, preventing effective digestion by enzymes. If we can understand better on how the plant sugars are arranged in the cell walls, we can select plants and match them with the most appropriate enzymes for more effective biofuel production. Improving the properties of lignin in lignocellulosic biomass will make it easier to produce biofuel (or bioenergy) from this material without detrimental effects on the yield or quality of the crop. Lignin is a strengthening and waterproofing polymer that encrusts the sugar-based polymers in plant cell walls, making them hard to access for biofuel production. Lignin and its by-products are also toxic to microorganisms used in fermentation. Feedstocks rich in lignocellulose require treatment with acids, alkalis or steam explosion methods to hydrolyse hemicellulose and break down lignin, enabling access to the cellulose by enzymes. The alternative process is to combine lignocellulosic enzyme system for one step

recovery of biosugars form lignocellulosic biomass with efficient removal of lignin.

To verify the efficiency of the technology, the next step is to implement all of these improvements in a pilot-scale process with all steps integrated into a continuous pilot plant. The simplified schematic diagram of all processes is shown in **Figure 12**. This will provide better data for assessment and for scale-up to a demo- or full-scale process. It will also give better information on how various pretreatment conditions will affect all the other processing steps, i.e. enzymatic hydrolysis, fermentation, downstream processing (extraction and purification) as well as product and co-product quality. Finally the conceptual of net energy output for biofuel production from lignocellulosic biomass through biorefining should be defined.

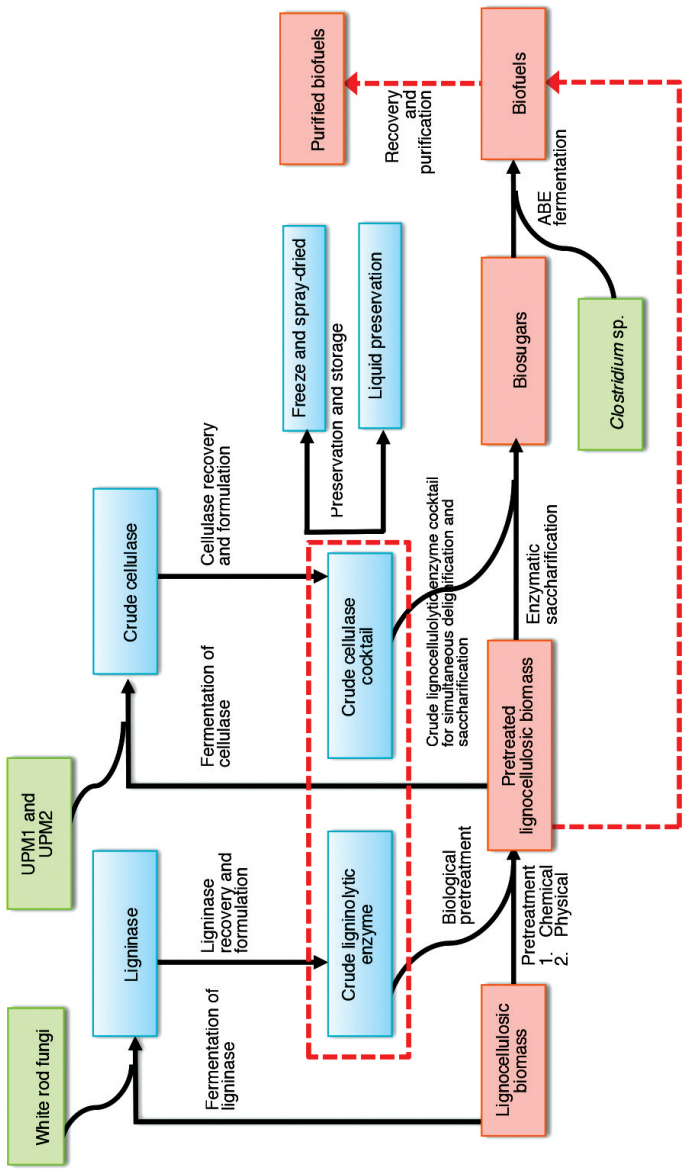

Figure 12 Schematic diagram of biofuels production from lignocellulosic biomass

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BIOGRAPHY

Professor Dr Suraini Abd-Aziz was born in the district hospital of Pontian, Johore on 6th January 1969. She is happily married to Professor Ir Dr Shahrir Abdullah and blessed with 3 daughters. Professor Suraini is currently Deputy Dean (Academic and Student Affairs) of the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia. She graduated with a BSc (Honours) Clinical Biochemistry from Universiti Kebangsaan Malaysia in 1992, MSc (Biochemical Engineering) from University of Wales Swansea, United Kingdom in 1994 and PhD (Biochemical Engineering) from University of Wales Swansea, United Kingdom in 1997.

Professor Suraini began her career as a Research Officer at SIRIM Berhad in March 1997 since her PhD study was under the JPA's Federal Scholarship scheme and Pre-Service Employment Scheme by SIRIM Berhad (SIRIM-ADB Attachment scheme). However, due to her deep interest in teaching as well as research, she resigned from SIRIM Berhad in August 1997.

Her academic career started in August 1997 as a lecturer at the Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia. Apart from teaching and research, she was actively involved in administration duties and community services. As a result, she has been conferred numerous awards since year 2000. Of particular note was her selection to be the first Malaysian and South-east Asian to win the prestigious UNESCO-L'Oreal Co-Sponsored Fellowship for Young Women in Life Sciences 2001 for Asia and The Pacific region. The award was given based on the quality of research proposal submitted and its significant contribution to humanity. Following that in 2002, she was awarded the Young Asian Biotechnology Prize by the Bioscience and Bioengineering Society, Japan on her contribution

to the development of success research collaboration with Japan. She was promoted to Associate Professor in 2002 and Professor in 2011.

In research, she has developed her research interest in the field of Industrial and Environmental Biotechnology. Apart from being the project leader of her own research grants, she has also actively contributed to other projects in her capacity, striving to become an expert in enzyme technology, fermentation technology, bioprocess engineering and environmental biotechnology. With the establishment of an active research group together with Prof. Dr Mohd Ali Hassan in Environmental Biotechnology, she had and is currently supervising and co-supervising over 75 post-graduate students, i.e. 23 PhD students and 52 MSc students. Moreover, she has also supervised more than 100 undergraduate students undertaking final year research projects leading for the BS (Biotechnology) degree. As recognition to her expertise in the above mentioned field, she has been appointed as an external examiner of MS and PhD theses for other universities including Universiti Kebangsaan Malaysia (UKM), Universiti Teknologi Malaysia (UTM), Universiti Malaya (UM), International Islamic University of Malaysia (IIUM), Universiti Sains Malaysia (USM), Universiti Malaysia Pahang (UMP), Universiti Malaysia Sabah (UMS) and Universiti Malaysia Sarawak (UNIMAS). She was also selected as internal examiner and chairman for postgraduate students' oral examination in UPM as well as being requested to be a reviewer for several cited journal papers and books, panel for e-Science application, panel for Techno-fund application as well as the panel for National Science Fellowship application. With a *h*-Index of 16 (total citations of 831), she has published more than 100 cited journals internationally and more than 100 proceedings internationally and nationally.

Looking back on her achievements and success, her main ambition and dream is to further develop the field of her research interest, to establish a research network in the related field in Malaysia and the rest of the world through collaboration with other scientists, to produce world class research output, and finally to be a renowned Professor and well-known scientist to make UPM and Malaysia proud.

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Suraini Abd. Aziz

LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
   The Challenge to Communication Research in Extension
   22 July 1989

2. Prof. Ir. Abang Abdullah Abang Ali
   Indigenous Materials and Technology for Low Cost Housing
   30 August 1990

3. Prof. Dr. Abdul Rahman Abdul Razak
   Plant Parasitic Nematodes, Lesser Known Pests of Agricultural Crops
   30 January 1993

4. Prof. Dr. Mohamed Suleiman
   Numerical Solution of Ordinary Differential Equations: A Historical Perspective
   11 December 1993

5. Prof. Dr. Mohd. Ariff Hussein
   Changing Roles of Agricultural Economics
   5 March 1994

6. Prof. Dr. Mohd. Ismail Ahmad
   Marketing Management: Prospects and Challenges for Agriculture
   6 April 1994

7. Prof. Dr. Mohamed Mahyuddin Mohd. Dahan
   The Changing Demand for Livestock Products
   20 April 1994

8. Prof. Dr. Ruth Kiew
   Plant Taxonomy, Biodiversity and Conservation
   11 May 1994

9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
   Engineering Technological Developments Propelling Agriculture into the 21st Century
   28 May 1994

10. Prof. Dr. Shamsuddin Jusop
    Rock, Mineral and Soil
    18 June 1994

11. Prof. Dr. Abdul Salam Abdullah
    Natural Toxicants Affecting Animal Health and Production
    29 June 1994

12. Prof. Dr. Mohd. Yusof Hussein
    Pest Control: A Challenge in Applied Ecology
    9 July 1994

13. Prof. Dr. Kapt. Mohd. Ibrahim Haji Mohamed
    Managing Challenges in Fisheries Development through Science and Technology
    23 July 1994

14. Prof. Dr. Hj. Amat Juhari Moain
    Sejarah Keagungan Bahasa Melayu
    6 August 1994

15. Prof. Dr. Law Ah Theem
    Oil Pollution in the Malaysian Seas
    24 September 1994

16. Prof. Dr. Md. Nordin Hj. Lajis
    Fine Chemicals from Biological Resources: The Wealth from Nature
    21 January 1995

17. Prof. Dr. Sheikh Omar Abdul Rahman
    Health, Disease and Death in Creatures Great and Small
    25 February 1995

18. Prof. Dr. Mohamed Shariff Mohamed Din
Fish Health: An Odyssey through the Asia - Pacific Region
25 March 1995

19. Prof. Dr. Tengku Azmi Tengku Ibrahim
Chromosome Distribution and Production Performance of Water Buffaloes
6 May 1995

20. Prof. Dr. Abdul Hamid Mahmood
Bahasa Melayu sebagai Bahasa Ilmu-Cabaran dan Harapan
10 June 1995

21. Prof. Dr. Rahim Md. Sail
Extension Education for Industrialising Malaysia: Trends, Priorities and Emerging Issues
22 July 1995

22. Prof. Dr. Nik Muhammad Nik Abd. Majid
The Diminishing Tropical Rain Forest: Causes, Symptoms and Cure
19 August 1995

23. Prof. Dr. Ang Kok Jee
The Evolution of an Environmentally Friendly Hatchery Technology for Udang Galah, the King of Freshwater Prawns and a Glimpse into the Future of Aquaculture in the 21st Century
14 October 1995

24. Prof. Dr. Sharifuddin Haji Abdul Hamid
Management of Highly Weathered Acid Soils for Sustainable Crop Production
28 October 1995

25. Prof. Dr. Yu Swee Yean
Fish Processing and Preservation: Recent Advances and Future Directions
9 December 1995

26. Prof. Dr. Rosli Mohamad
Pesticide Usage: Concern and Options
10 February 1996

27. Prof. Dr. Mohamed Ismail Abdul Karim
Microbial Fermentation and Utilization of Agricultural Bioresources and Wastes in Malaysia
2 March 1996

28. Prof. Dr. Wan Sulaiman Wan Harun
Soil Physics: From Glass Beads to Precision Agriculture
16 March 1996

29. Prof. Dr. Abdul Aziz Abdul Rahman
Sustained Growth and Sustainable Development: Is there a Trade-Off 1 or Malaysia
13 April 1996

30. Prof. Dr. Chew Tek Ann
Sharecropping in Perfectly Competitive Markets: A Contradiction in Terms
27 April 1996

31. Prof. Dr. Mohd. Yusuf Sulaiman
Back to the Future with the Sun
18 May 1996

32. Prof. Dr. Abu Bakar Salleh
Enzyme Technology: The Basis for Biotechnological Development
8 June 1996

33. Prof. Dr. Kamel Ariffin Mohd. Atan
The Fascinating Numbers
29 June 1996

34. Prof. Dr. Ho Yin Wan
Fungi: Friends or Foes
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