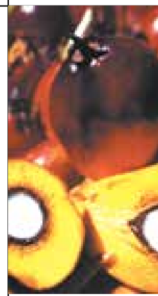

Lignocellulose-derived Sugars from Oil Palm Biomass

Nurul Adela Bukhari*; Soh Kheang Loh*; Nasrin Abu Bakar* and Jamaliah Md Jahim**

INTRODUCTION

Lignocellulosic biomass is an interesting option as a source of fermentable sugars for the production of biofuels and biochemicals considering its abundant availability and low-cost compared to commercially available refined sugars. One such major lignocellulosic biomass available in Malaysia is empty fruit bunches (EFB) which is a by-product from the palm oil milling process. Besides, the oil palm plantations also generate a large amount of oil palm trunk (OPT) and oil palm frond (OPF) during replanting and pruning. The annual production of EFB, OPT and OPF is approximately 84.23 million tonne (dry basis) a year (*Table 1*) from 5.74 million hectare of oil palm planted area (MPOB, 2016). This huge amount (*i.e.* 7 million t of EFB, 21.4 million t of OPT and 55.8 million t of OPF) implies that oil palm biomass is a readily available feedstock to provide intermediate platforms (xylose, C5 and glucose, C6 sugars) for the production of biofuels and biochemicals.

The National Biomass Strategy (NBS) 2020 was initiated in November 2011 to assess how Malaysia can gain more revenue from its natural resources including the palm oil industry through utilisation of the associated biomass for higher value downstream applications. A year later, the Bioeconomy Transformation Programme (BTP) was also launched with the same objectives but with broader coverage. The production of biofuels and biochemicals utilising oil palm biomass, in this regard, is in line with these two initiatives in strengthening Malaysia as a regional

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TABLE 1. AVAILABILITY OF LIGNOCELLULOSIC OIL PALM BIOMASS

Production site	Residue type	Biomass (t ha^{-1}, dwb)	Estimated amount (million t, dwb)	Remarks
Mill	Empty fruit bunch (EFB)	1.2	7.03	Based on annual fresh fruit bunch (FFB) yield
Plantation	Oil palm trunk (from replanting activity)	74.5*	21.38	Based on 5% estimated oil palm planted area due for replanting
	Oil palm frond (from replanting activity)	14.5*	4.16	Based on 5% estimated oil palm planted area due for replanting
	Oil palm frond (from pruning activity)	12*	51.66	Based on 75% of oil palm planted area pruned per annum

Note: *Sukiran *et al.*, (2017).

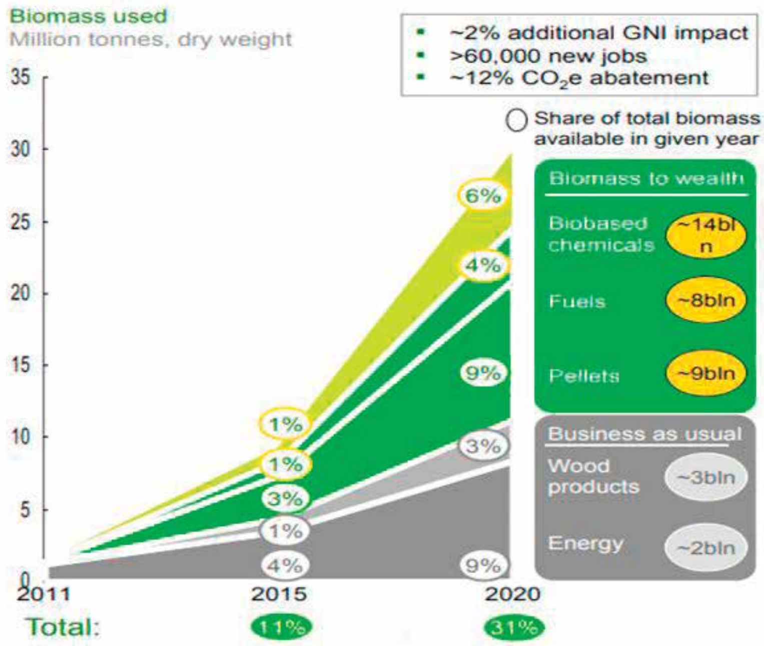

Figure 1. The National Biomass Strategy (NBS) 2020 identifies specific opportunities to create high-value industries by mobilising 30 million t biomass by 2020.

Source: *NBS 2020.*

bioeconomy hub by 2020. The biggest opportunity with tremendous prospect in the future is perhaps the bio-based chemicals with a forecasted global market size of ~14 billion by 2020, followed by fuels (~8 billion) and pellets (~9 billion) (*Figure 1*). Several such specific bio-based industries may advance as potential key strength to the nation compared to those conventional wood-based uses.

OIL PALM BIOMASS

The oil palm biomass is a lignocellulosic biomass consists primarily of cellulose, hemicellulose and lignin in an intricate structure, which is recalcitrant to decomposition. Cellulose $(C_6H_{10}O_5)x$, the main component of oil palm biomass is a linear polymer of glucose with sophisticated orientation of the linkages and additional hydrogen bonding making the polymer rigid, strong and difficult to break (Abdel-Rahman *et al.*, 2011). The polysaccharide is broken down to free sugar molecules by the addition of water via hydrolysis (Hamelinck *et al.*, 2005). This process is also known as saccharification and the product, glucose is a C6 sugar.

On the other hand, hemicellulose $(C_5H_8O_4)m$, located in secondary cell walls, consists of short, highly branched chains of various pentoses (*i.e* xylose and arabinose) and hexoses (*i.e* mannose, galactose and glucose). Hemicellulose is relatively easy to be hydrolysed compared to cellulose due to their weaker amorphous and branched structures (Balat, 2011).

Besides, lignin $[C_9H_{10}O_3(OCH_3)0.9\text{-}1.7]n$ - the major non-carbohydrate component – is a highly complex compound with three dimensional cross-linked polyphenolic structure. It is closely attached between the cell wall of the cellulose and the hemicellulose; hence is responsible for the remarkable strength of the cell wall and the plant as a whole. As such, lignin poses a major drawback when used as a lignocellulosic biomass in fermentation, as it is resistant to chemical and biological degradation (Taherzadeh and Karimi, 2008).

BIOCHEMICAL CONVERSION PROCESS

Various process configurations are possible to convert biomass into biochemicals and biofuels; the most typical being biochemical conversion consisting of four key steps:

1. Pretreatment: A mechanical or thermochemical pretreatment step to reduce the size of the feedstock and break up the lignocellulosic structure.
2. Hydrolysis: A chemical or enzymatic reaction that converts the cellulose and hemicellulose to monomeric sugars.
3. Fermentation: Microorganisms ferment the sugars to the targeted products.
4. Separation and purification of the targeted products.

Feedstock pretreatment and hydrolysis

As cellulose and hemicellulose are associated with lignin, pretreatment of lignocellulose of oil palm biomass is crucial for achieving effective hydrolysis of substrates. Although the cost of biomass pretreatment is high, the cost associated with no pretreatment is even higher. The main goals of pretreatment are to remove the lignin, disrupt the hemicellulose structure, reduce the crystallinity and degree of polymerisation of cellulose, and increase the porosity of the substrates which will then aid in the subsequent access by hydrolytic enzymes (Kumar *et al.*, 2009a) for sugars production.

Pretreatment methods include physical (grinding and milling, microwave and extrusion), chemical (alkali, acid, organosolv, ozonolysis and ionic liquid), physicochemical (steam explosion, liquid hot water, ammonia fibre explosion, wet oxidation and CO_2 explosion) and biological pretreatment. So far, the pretreatment methods developed extensively for oil palm biomass include ethanolic hot compressed water (Goh *et al.*, 2010), hot compressed water (Goh *et al.*, 2012), ultrasonication (Robiah *et al.*, 2010), steam explosion/ autohydrolysis (Sabiha-Hanim *et al.*, 2015), ionic liquid (Tan *et al.*, 2011), alkaline (Indera Luthfi *et al.*, 2016; Jung *et al.*, 2011; Piarpuzan *et al.*, 2011), acid (Mohd Asyraf *et al.*, 2011) and biological pretreatment (Rahman *et al.*, 2011). Among these, dilute acid pretreatment is probably the most commonly applied method to produce sugars from oil palm biomass. The establishment of suitable pretreatment method allows efficient recovery of fermentable sugars from the digestible structural carbohydrates in oil palm biomass.

Enzymatic hydrolysis or saccharification of biomass is applied to yield fermentable sugars mainly from the cellulose of the pretreated biomass. This is commonly done using cellulase in order to depolymerise the cellulose to glucose. For efficient saccharification, a synergistic reaction of the three classes of cellulolytic enzymes is required *i.e.* Endo-β-1,4glucanases (EG; EC 3.2.1.3), Exo-β-1,4-glucanases or cellobiohydrolases (CBH; EC 3.2.1.91) and β-glucosidases (β-G: EC 3.2.1.21) (Kumar *et al.*, 2009b).

Biocatalysis

Microorganisms metabolise sugars derived from lignocellulosic biomass to a number of desired products *via* fermentation. For examples, ethanol is produced using *Saccharomyces cerevisiae* and *Zymomonas mobilis*, butanol by *Clostridium acetobutylicum*, lactic acid by *Lactobacillus sp.*, succinic acid by *Actinobacillus succinogenes*, *etc.* These wild type microorganisms are capable of producing biochemicals of interest at relatively high concentrations. *Figure 2* shows the potential bioconversion of oil palm biomass into biochemicals and biofuels.

METHODOLOGY

In this study, fermentable sugars were produced from EFB, OPT and OPF using acid and enzymatic hydrolyses. Initially they were pretreated with diluted sulphuric acid at different concentration (0.1%-2.0%, v/v), temperature (105°C-125°C) and reaction time (15-120 min). The treated materials were subsequently analysed for sugars content. The pretreated solids were subsequently hydrolysed with commercial cellulases to obtain glucose.

The enzymatic hydrolysis was performed using a commercial cellulase *i.e.* Celluclast 1.5 litre derived from *Trichoderma reesei* (Novozyme A/S, Bagsvaerd, Denmark) for EFB and F3 UKM-enzyme derived from genetically modified strain of *Pichia pastoris* for OPT and OPF. Besides, addition of several surfactants *i.e.* Tween 20 (polyoxyethylene sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monoleate) and Triton X-100 ($C_{34}H_{62}O_{11}$) was conducted to examine if they can enhance glucose production during enzymatic hydrolysis. The optimum pretreatment and enzymatic hydrolysis conditions for the

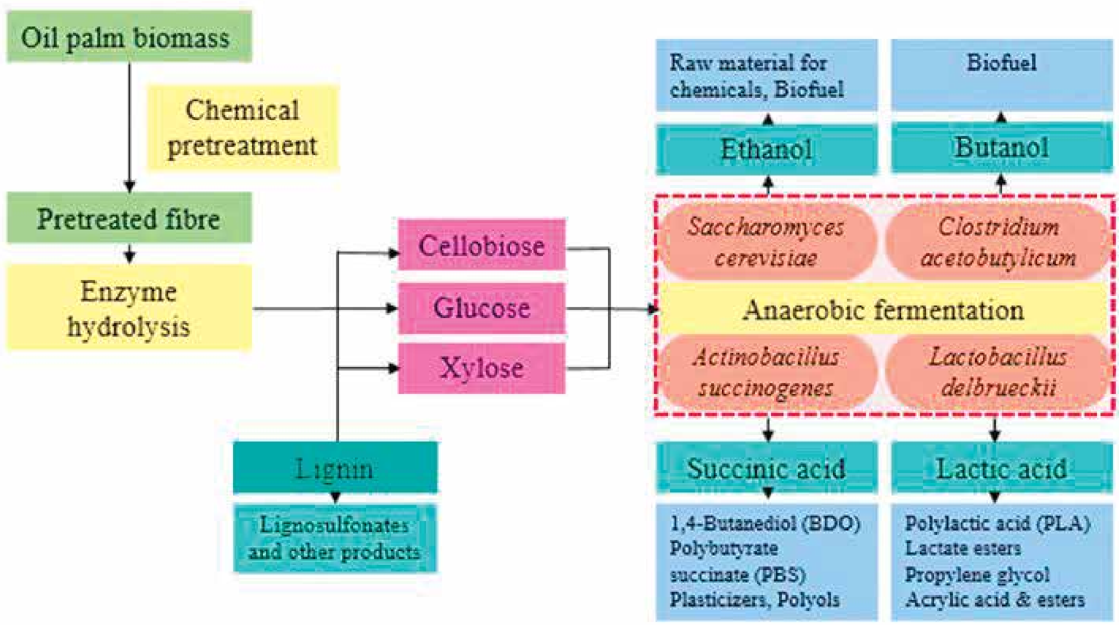

Figure 2. Schematic representation of biocatalysis of oil palm biomass to biofuels and biochemical (adapted from Adsul et al., 2011).

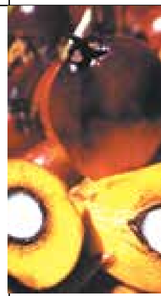

TABLE 2. PRETREATMENT AND ENZYMATIC HYDROLYSIS CONDITIONS FOR EMPTY FRUIT BUNCH (EFB), OIL PALM TRUNK (OPT) AND OIL PALM FROND (OPF)

Biomass	Pretreatment condition	Enzymatic hydrolysis condition
EFB*	1% (v/v) sulphuric acid, T = 125°C, t = 90 min, particle size = 91-106 μm	30 FPU U/g Celluclast 1.5 L + 1% (v/v) Cellic Ctec + 0.5% (v/v) Triton X-100, T = 50ºC, t = 72 hr, agitation = 150 rpm
OPT	1% (v/v) sulphuric acid, T = 120°C, t = 90 min, particle size = 1-10 mm	50 FPU U/g F3 UKM-enzyme (Celluclast + Cellic Ctec + xylanase) + 0.1% (v/v) Triton X-100, T = 50ºC, t = 48 hr, agitation = 155 rpm
OPF	2% (v/v) sulphuric acid, T = 120°C, t = 90 min, particle size = 1-10 mm	50 FPU U/g F3 UKM-enzyme (Celluclast + Cellic Ctec + xylanase), T = 50ºC, t = 72 hr, agitation = 155 rpm

Note: *Nurul Adela *et al.* (2015).

three types of oil palm biomass used in this study are summarised in *Table 2*.

RESEARCH FINDINGS

The total carbohydrate contents (C5 + C6) of the three types of oil palm biomass (*i.e.* EFB, OPT and OPF) were determined. They ranged from 71.1%-73.6% (*Table 3*), showing their suitability as feedstocks for bioconversion. Result indicated that C5 and C6 sugars were released as the main carbon source after acid pretreatment followed by enzymatic hydrolysis.

From the initial acid hydrolysis process, the maximum xylose yields attainable from EFB, OPT and OPF were 27.7 g litre^{-1}, 18.1 g litre^{-1} and 20.2 g litre^{-1}, respectively. It was found that higher yields would be realised with increasing reaction time and temperature. Normally, the effect of temperature on xylose yield was more significant than those of acid concentration and reaction time (unpublished data). From this chemical pretreatment, the highest conversion efficiency of hemicellulose to xylose were 95.5%, 70.0% and 70.4% for EFB, OPT and OPF, respectively. The high conversion efficiency in EFB might be due to the smaller particles size used in the experiment (*i.e.* 91-106 μm); thus larger surface area were exposed to the reactant, providing better contact for bioconversion.

The addition of non-ionic surfactant *i.e.* Triton X-100 during enzymatic hydrolysis was capable of modifying the cellulose surface property, minimising the irreversible binding of cellulase onto cellulose (Sun and Cheng, 2002) and reduction of non-specific and irreversible adsorption of cellulase onto lignin (Seo *et al.*, 2011). In this study, the addition of surfactant increased the glucose yields of EFB and OPT by 1.8-fold and 1.6-fold, respectively (unpublished data).

Overall, from the enzymatic hydrolysis, the highest conversion efficiency of cellulose to glucose were 63.7%, 59.0% and 49.0% for EFB, OPT and OPF, respectively. It was found that EFB produced the highest yield followed by OPF and OPT; with total sugars conversion of 79.0%, 66.0% and 62.0%, respectively (*Table 4*).

CONCLUSION

This study indicated that oil palm biomass has huge potential as a feedstock for downstream biofuels and biochemicals production. The results obtained concurred with the government initiatives highlighting on bioconversion of oil palm biomass into higher value-added products. The bioconversion route in producing biofuels and biochemicals from oil palm biomass may create new business to potentially transform the Malaysian palm oil industry in the future.

TABLE 3. COMPOSITION OF EMPTY FRUIT BUNCH (EFB), OIL PALM TRUNK (OPT) AND OIL PALM FROND (OPF)

Chemical composition (wt. %)	EFB*	OPT	OPF
Cellulose	44.53 ± 0.06	30.86 ± 3.29	42.43 ± 2.63
Hemicellulose	29.05 ± 1.48	25.84 ± 4.61	28.71 ± 2.42
Lignin	23.45 ± 1.48	24.29 ± 5.82	26.20 ± 2.88
Ash	2.98 ± 0.07	2.29 ± 0.15	3.03 ±0.55
Others	-	16.72 (starch)	-
Total carbohydrate	**73.58**	**73.42**	**71.14**

Note: * Nurul Adela *et al.* (2014).

TABLE 4. YIELD OF SUGARS FROM DILUTE ACID FOLLOWED BY ENZYMATIC HYDROLYSIS OF EMPTY FRUIT BUNCH (EFB), OIL PALM TRUNK (OPT) AND OIL PALM FROND (OPF)

Amount of oil palm biomass (1000 kg, dwb)	EFB*	OPT	OPF
Hemicellulose content (kg)	290.5	258.4	287.1
Hemicellulose conversion (%)	95.5	70.0	70.4
Xylose (kg)	277.3	181.0	202.0
Glucose from hemicellulose (kg)	20.5	91.0	62.0
Cellulose content (kg)	445.3	308.6	424.3
Cellulose conversion (%)	63.7	59.0	48.6
Glucose from cellulose (kg)	283.6	182.0	206.2
Total sugars from 1000 kg oil palm biomass (kg)	**581.4**	**454.0**	**470.2**
Total sugars conversion from total carbohydrate	**79%**	**62%**	**66%**

Note: *Nurul Adela *et al.* (2014; 2015).

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of Malaysian Palm Oil Board (MPOB) for permission to publish this article. Thanks are also extended to the Malaysia Genome Institute (MGI) for providing the enzyme under research fund from Ministry of Agriculture No. TF0310F086. The technical assistances provided by the interns and the staff of the Energy and Environment Unit of MPOB are also deeply appreciated.

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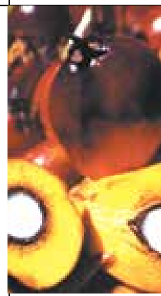

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