EFFECT OF HIGH-PRESSURE STEAM TREATMENT ON ENZYMATIC SACCHARIFICATION OF OIL PALM EMPTY FRUIT BUNCHES

Azhari Samsu Baharuddin,* Noor Seribainun Hidayah Md Yunos, Nik Anis Nik Mahmud, Rabitah Zakaria, and Khairul Faezah Md Yunos

The effectiveness of high-pressure steam treatment (HPST) with various treatment temperatures (170, 190, 210, and 230 ^{o}C) on the enzymatic hydrolysis yield of oil palm empty fruit bunches (OPEFB) was successfully investigated. Analysis of the compositions of raw and treated OPEFB showed that significant changes occurred after the HPST was performed. Scanning electron microscopy (SEM) analysis showed that the treated OPEFB gave better results in removing the silica bodies as compared to the untreated OPEFB. This analysis was in agreement with FTIR results, which revealed a significant decrease in the content of hemicelluloses after HPST. During saccharification, the amount of sugar produced was higher for treated OPEFB than untreated OPEFB. Thus, the results suggest that HPST can be applied as an alternative treatment method for the alteration of OPEFB structure and to enhance of the digestibility of the biomass, therefore improving enzymatic hydrolysis.

Keywords: High-pressure steam; Oil palm empty fruit bunches; Enzymatic hydrolysis

Contact information: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor, Malaysia.
**Corresponding author: azharis@eng.upm.edu.my*

INTRODUCTION

Currently, 55.73 million tons of oil palm biomass are generated in Malaysia, of which oil palm empty fruit bunches (OPEFB) contribute 17.0 million tons. That number is expected to increase in the future (Shuit *et al.* 2009). Nowadays, Malaysia encourages the production of bioethanol from biomass waste, especially from oil palm empty fruit bunches (OPEFB), in order to increase the resources of ethanol production and to reduce the cost. According to Baharuddin *et al.* (2010), lignocellulosic material in OPEFB is mainly composed of holocellulose (cellulose and hemicelluloses) and lignin with a high potential to be the substrate for the production of high value added bioproducts such as biosugar, bioethanol, biogas, and others. Bioethanol is an alternative source of fuel and its industry is relatively new. With the increasing worldwide demand for fuel and energy, it has been universally recognized that alternative, sustainable sources for transport fuel are needed to ensure the security and longevity of supply.

The application of cellulose to produce bioethanol is hampered by the high cost and the low efficiency of pre-treatment methods. Several methods, such as acid hydrolysis and enzymatic saccharification, have been proposed to hydrolyse lignocellulosic material into reducing sugar. However, the saccharification process is

limited by several factors, such as the crystalline structure of cellulose, the protective sheath of lignin, the presence of hemicellulose around the cellulose, the moisture content, and the available surface area (Chang and Holtzapple 2000; Laureano-Perez *et al.* 2005). Due to these circumstances, pre-treatment of the material is essential to improve the accessibility of enzyme to lignocellulosic materials in order to obtain a high overall yield of sugar production.

Numerous pre-treatment methods have been reported in order to enhance the digestibility of lignocellulosic material such as physical pre-treatment (milling, grinding, hydrothermolysis), chemical pre-treatment (acid, alkaline), physio-chemical pre-treatment (steam explosion, steam treatment, ammonia fiber explosion), and biological pre-treatment techniques. Depending on the biomass composition and operating conditions, different pre-treatment techniques may be applied. Alkaline pre-treatment is more often used for agricultural residues and herbaceous crops than for wood materials. However, most of the chemical pre-treatments are toxic, corrosive, and can be harmful to the environment. There is also a risk of the formation of inhibiting compounds (Singh *et al.* 2009), making it less attractive than other pre-treatment techniques. On the contrary, biological treatments, such as fungal pre-treatment, are advantageous in that the raw materials needed are inexpensive and the operating conditions are mild. Hence, no sugar degradation products are formed (Taniguchi *et al.* 2005). Nevertheless, the disadvantages of biological pre-treatment include long pre-treatment times and the fact that consumption of carbohydrates by the fungus will reduce the yield of bio-ethanol (Balan *et al.* 2008).

Amongst the various pre-treatment methods, mechanical pre-treatment is the most common physical treatment. Mechanical treatment, such as milling, is done to increase the surface area for enzyme interaction. The milling process can reduce the cellulose crystallinity and the degree of polymerization and also causes shearing of the biomass (Palmowski and Muller 1999). Despite the advantages, this method requires a large amount of energy (Ramos 2003) and therefore it is not economically feasible as a pre-treatment method, due to increasing energy costs. Steam explosion is the most widely used method for the pre-treatment of lignocellulosic materials (Sun and Cheng 2002). The objective of steam explosion is to solubilize the hemicellulose to make the cellulose more accessible for enzymatic hydrolysis. However, Zabihi *et al.* (2010) stated that the pre-treatment of lignocellulosic materials by steam explosion alone is less effective compared to steam explosion after soaking with acetic acid or ethanol. The priority of this research is to provide a method that does not involve any toxic chemicals, especially the release of hazardous chemicals into the environment.

Another promising pre-treatment method is high-pressure steam pre-treatment (HPST). High-pressure steam treatment could be the ideal pre-treatment method for the production of bio-sugar because the process involves a lower environmental impact, less hazardous process chemicals, and, most importantly, steam is readily available at all palm oil mills in Malaysia. A previous study has proven that high-pressure steam treatment affects the chemical composition of OPEFB, polysaccharide conversion, sugar production, and the morphology of OPEFB structure (Shamsudin *et al.* 2012). Although many studies have been done to identify the most suitable pre-treatment for the bioconversion of sugar, it is a great challenge to find the most reliable one, especially for

large-scale purposes. Therefore, the main objective of this research is to determine the optimum pretreatment conditions of OPEFB using high pressure steam treatment (HPST) for the production of sugar.

EXPERIMENTAL

Materials

Oil palm empty fruit bunches (OPEFB) were obtained from Seri Ulu Langat Palm Oill Mill (Dengkil Selangor, Malaysia). About 10 g of OPEFB was dried at 60 °C for 24 hours prior to the treatment.

Pre-Treatment Using High-Pressure Steam

The high-pressure steam treatment (HPST) of OPEFB was conducted based on methods described by Bahrin *et al*. (2012) using a 500 mL high-pressure autoclave (START 500, Nito Kuatsu, Co. Ltd, Japan) equipped with temperature and pressure control systems. It has the ability to reach a temperature up to 250 °C and a pressure of 9.4 MPa. The treatment temperature and pressures were 170 °C and 0.82 MPa, 190 °C and 1.32 MPa, 210 °C and 2.03 MPa, and 230 °C and 3.00 MPa, and the treatment was carried out for 2 minutes.

Scanning Electron Microscope (SEM)

A scanning electron microscope (S-3400N, Hitachi, Japan) was used to analyze the morphological structure of the superheated steam-treated OPEFB. The samples were mounted on an aluminum stub using double-sided adhesive tape and were sputter-coated (E-1010, Hitachi, Japan) with platinum prior to the morphological examination. The SEM micrographs were obtained at acceleration voltage of 15 to 25 kV.

Fourier Transform Infrared (FTIR)

FTIR experiments were performed using Spectrum^{TM} GX, 2000R (Perkin Elmer USA) at 500 to 4000 cm^{-1} wave numbers. The instrument was operated at 4 cm^{-1} resolution and samples were subjected to 16 scans. An attenuated total reflectance (ATR) was applied to obtain information about the surface modification of the OPEFB samples.

Chemical Composition Analysis (CHL)

The chemical composition of cellulose, hemicellulose, and lignin of HPST OPEFB were assayed for acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) (Goering and Van Soest 1970). The percentages of cellulose, hemicelluloses, and lignin were calculated using the equations below:

$$\text{Cellulose (\%)} = \text{ADF- ADL} \quad (1)$$

$$\text{Hemicellulose (\%)} = \text{NDF- ADF} \quad (2)$$

$$\text{Lignin (\%)} = \text{ADL} \quad (3)$$

Saccharification of OPEFB

The enzyme used was Celluclast 1.5 L with an enzyme loading of 2.5, 5.0, 7.5, and 10.0% v/v. Sodium azide (0.02% w/v) was added to avoid bacterial or fungal contamination. Saccharification of the OPEFB was performed in duplicate with 5% (w/v) of substrate in 50 µM of sodium acetate buffer pH 5. Each enzyme concentration, containing 21.33, 42.66, 63.99, and 85.32 FPU of cellulase activity, was added to the solution for the conversion process. The process was carried out for 24 hours in an orbital shaking incubator (SI600-R, LabCompanion, Korea) with temperatures of 30, 40, and 50 °C and agitated at 200 rpm. Total reducing sugars was determined using dinitrosalicyclic acid (DNS) based on Miller's method (1959). The hydrolysis percentages were calculated according to equation described by Latif *et al.* (1994) as follows:

$$\text{Hydrolysis (\%)} = \frac{\text{[total reducing sugar (g/L) x 0.9 x 100]}}{\text{[Substrate (g/L) x potential sugar (g/g)]}} \quad (4)$$

$$\text{Hydrolysis Yield (g/g)} = \text{Product / Substrate} \quad (5)$$

RESULTS AND DISCUSSION

The chemical compositions of untreated and treated OPEFB with various treatment temperatures are summarized in Fig 1. The chemical composition analyses for both the untreated and the treated samples were conducted using Goering's method (Baharuddin *et al.* 2011). The initial composition of cellulose, hemicellulose, and lignin consisted of about 47.6, 28.1, and 13.1%, respectively. Increasing the pretreatment temperature from 170 °C to 210 °C increased the percentage ratio of cellulose content in the OPEFB from ± 50 to 70%. In addition, the composition of lignin also increased from 13.1% (raw) to 16.6% (210 °C). On the other hand, the hemicellulose content was dramatically reduced from 28.1% (raw) to 1.2% (230 °C). The disappearance of component in OPEFB material was corresponded to the percentage of weight (170^{o}C, 15.2%), (190^{o}C, 23.3%), (210^{o}C, 39.7%) and (230^{o}C, 46.8%). It shows a significant increment of weight loss as treatment temperature is increased. According to Gupta and Demirbas (2010), the degradation of hemicellulose parts is due to the generation of organic acid by hemicellulose's acetyl group cleavage, which hydrolyses some of the hemicellulose and alters the lignin structure, thereby increasing the percentage of cellulose. By nature, lignin provides rigidity, impermeability, and support to the plant structure (Hendriks and Zeeman 2009). Therefore, the presence of lignin in the cellular wall protects the structure of any lignocellulosic material during the pretreatment of OPEFB.

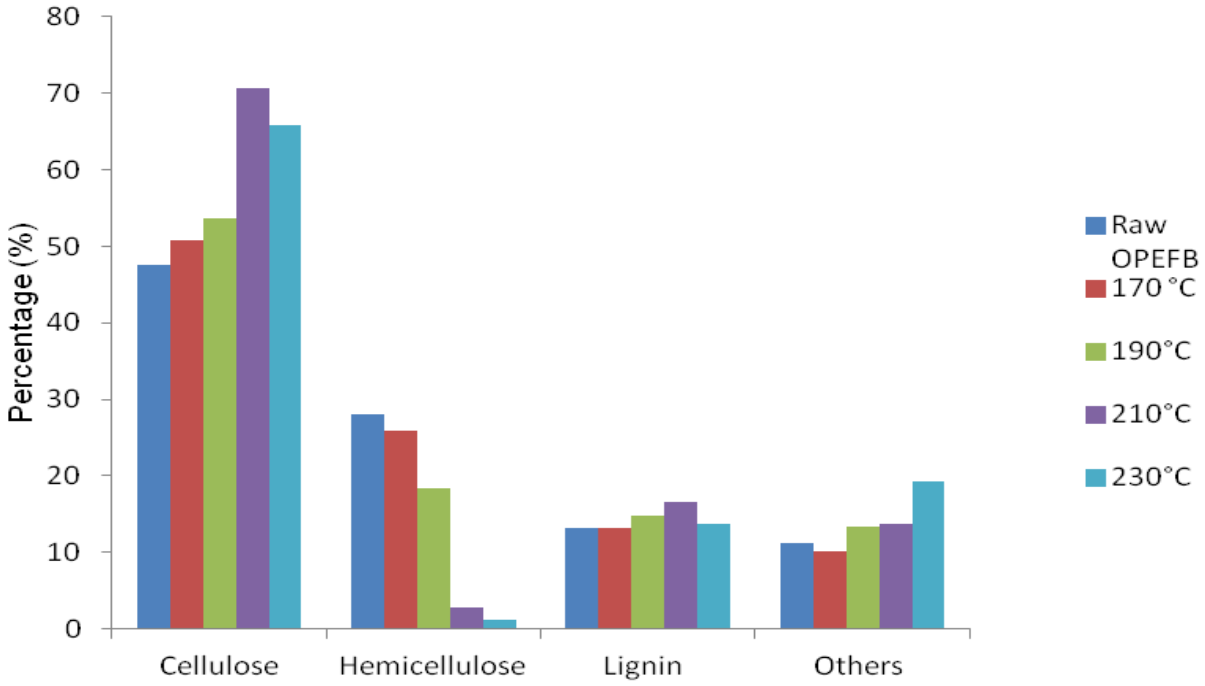

Fig. 1. Average compositions of elements in raw and high-pressure steam treated OPEFB at different temperatures

When steam temperature was increased from 210 °C to 230 °C, the percentage ratio of cellulose and lignin decreased from 70.6% to 65.9% and 16.6% to 13.7%, respectively. This phenomenon occurred because of the formation of thermally-converted products from cellulose and lignin at high temperature and steam conditions. Laser *et al.* (2002) reported that when the treatment temperature exceeded 220 °C during the 2 min pretreatment time, the formation of furfural and other compounds (probably soluble lignin compounds) occurred and inhibited the formation of ethanol. Thus, it was suggested that 210 °C/ 2.03 MPa was the optimum treatment temperature for the high-pressure steam treatment of OPEFB biomass.

Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) analysis was conducted on the untreated and the treated OPEFB at different temperatures to examine the effect of HPST on the OPEFB surface structure as shown in Fig. 2. From this figure, there are obvious differences between the untreated and the treated OPEFB with HPST. Initially, the untreated OPEFB (Fig. 2a) appeared rough and rigid, and the surface was embedded with silica bodies. This could due to the presence of a layer of matrix material such as lignin or waxes on the surface of substrate material which prevent the loss of water in most plants (Shamsudin *et al*. 2012).

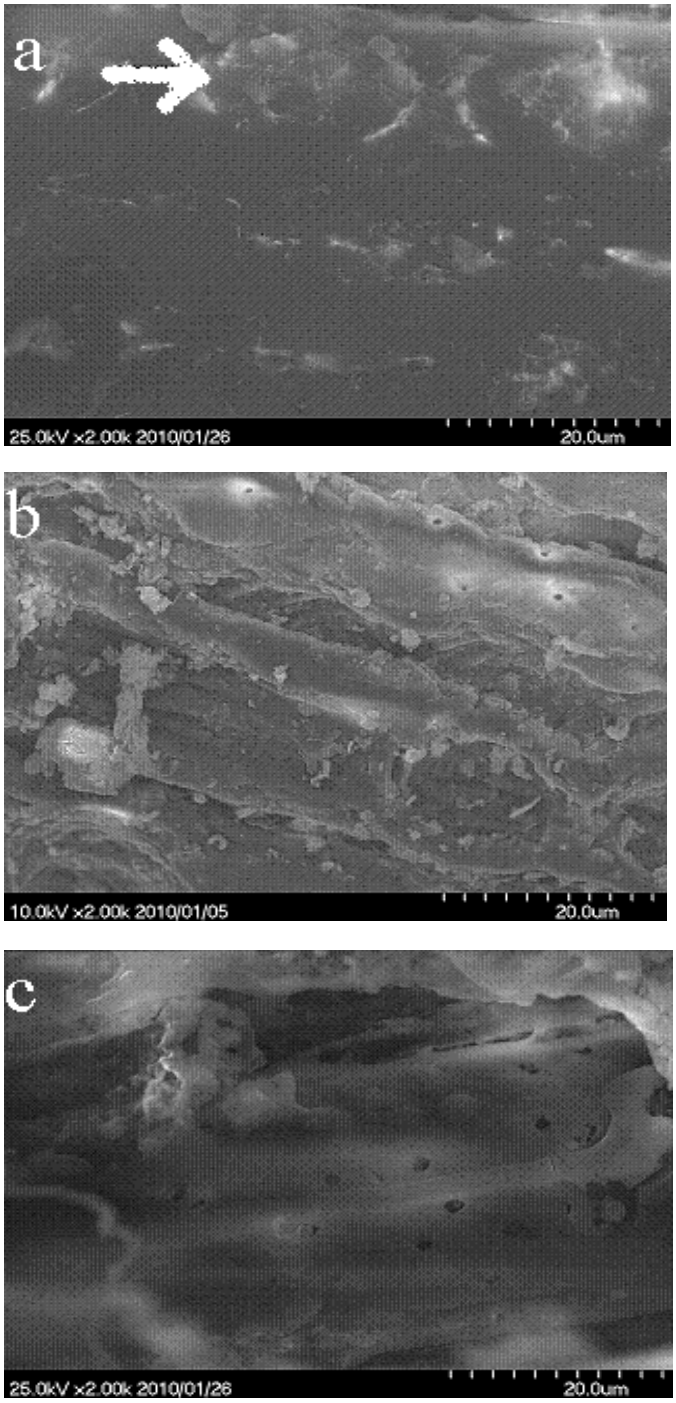

Fig. 2. SEM micrograph of raw OPEFB (a), high-pressure steam treated OPEFB at 210 °C (b), and at 230 °C (c). Arrow indicates silica bodies in the OPEFB strand

Meanwhile, for the sample treated at 210 °C (Fig. 2b), the SEM analysis revealed the existence of pores, which are visibly separated and cracked along the inner structure of the OPEFB. Furthermore, some of the silica bodies had been removed from the

OPEFB structure. This finding exhibits that there was sufficient energy to remove silica bodies and this result implied that HPST is an effective method for the modification of OPEFB outer surface. According to Neethirajan *et al.* (2009), the functions of silica bodies were to protect plant cell walls against fungal attack and to provide strength to the plant structure. However, the presence of silica bodies on the surface of OPEFB will hinder the enzymes from attacking the surface of the lignocellulosic structure.

Through SEM analysis it was found that the HPST had an effect on the lignocellulose in the OPEFB and increased the reactive area of the OPEFB surface structure. In addition, after steam treatment was performed, the porous cell walls were found to shrink and soften which then facilitated the penetration of enzyme and enhanced the accessibility for enzymatic hydrolysis (Shamsudin *et al.* 2012). As the treatment temperature was raised from 210 °C to 230 °C (Fig. 2c), there were noticeable differences in the physical structure of OPEFB due to the effects of such a high temperature. Moreover, some components of the OPEFB sample were almost completely degraded. In addition, it can be assumed that steam treatment at 210 °C/2.03 MPa significantly altered the structure of OPEFB without disrupting the presence of components in the OPEFB biomass.

FTIR Spectra Analysis

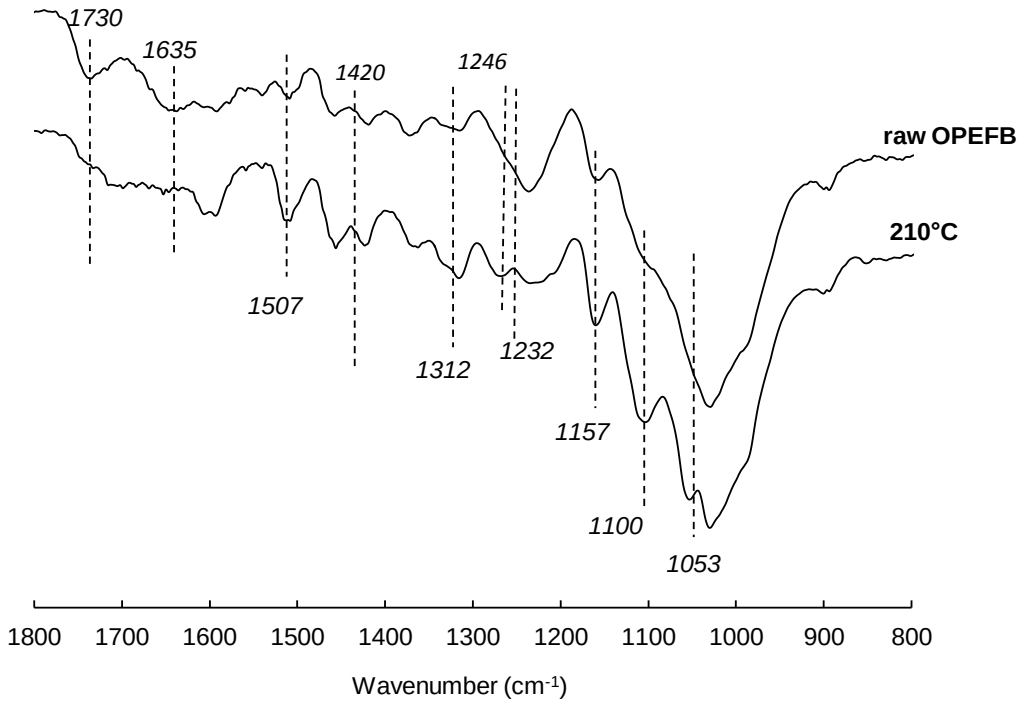

Fig. 3. FTIR spectra of raw and high-pressure steam treatment OPEFB for 210 °C

Figure 3 shows the result of the Fourier transform infrared analysis of the untreated and treated OPEFB sample at conditions of 210 °C and 2.03 MPa. The result of FTIR spectroscopy shows the most conspicuous changes at wave numbers 1730 cm^{-1}, 1635 cm^{-1}, 1246 cm^{-1}, 1232 cm^{-1}, and in the range 1000 to 1200 cm^{-1}.The band assignments according to the literature and band shifts are listed in Table 1. The absorption at 1730 cm^{-1} was attributed to the ester linkage of carboxylic acids, which gradually diminished after pre-treatment. It was reported that vC=O of acetyl and uronic esters corresponds to the hemicellulose component (Kristensen *et al.* 2008). Concurrently, the band at 1232 cm^{-1}, attributed to the vC=O of carboxyl group, corresponding to the hemicellulose component, also disappeared after pre-treatment (Smidt *et al.* 2005).

The bending mode of the absorbed water was recorded at 1635 cm^{-1}. The disappearance of this absorption indicates the preferential decomposition of the hemicellulose component. In addition, the adsorptions in the 1590 to 1600 cm^{-1} range were the characteristic bands of aromatic skeletal vibration that contribute to the lignin and cellulose components (Pandey 1999). The increased peak of this band shows that the amount of lignin and cellulose increased when the sample was pre-treated.

A peak at 1246 cm^{-1} indicates the presence of a structural carbohydrate such as cellulose (Wetzel *et al.* 2001). It was found that the band at 1246 cm^{-1} increases after pre-treatment. On the other hand, the presence of a sharp peak in the range of frequency between 1000 and 1200 cm^{-1} portrayed the C-O-C stretching and C-OH linkages, which were reported to be dominant in the cellulose and lignin components (Yang *et al.* 2007).

In this study, amongst the three lignocellulosic components, hemicellulose was the easiest component to decompose, with decomposition mainly occurring in the range 220 to 315 °C due to its branched structure, which formed amorphous regions. However, cellulose decomposition happens at the higher temperature range of 315 to 400 °C (Yang *et al.* 2007). The most difficult component to decompose was lignin. The decomposition happens slowly at a wide range of temperatures, from ambient conditions to 900 °C. Hubbe *et al*. (2010) reported that within the biomass component, lignin tends to decrease less compared to cellulose and hemicelluloses during composting. Due to these beneficial proven circumstances, high-pressure steam treatment can cause the alteration of the three structures in the OPEFB. This result is in agreement with cellulose, hemicellulose, and lignin analysis.

Saccharification of Treated OPEFB at Different Enzyme Loading

The profiles of reducing sugar yield during saccharification of untreated and treated OPEFB at various temperatures with different enzyme loading are shown in Fig 4. Each enzyme concentration contained 21.33, 42.66, 63.99, and 85.32 FPU of cellulose activity, respectively. It was found that when the saccharification of untreated OPEFB was performed, the yield of reducing sugar was the lowest (< 5 g/L). However, for treated OPEFB, the increase of reducing sugar yield was recorded from <5 g/L up to > 15 g/L in the treatment conditions of 170 °C to 210 °C of each cellulose activity. It can be seen that the maximum reducing sugar recorded was 22.1 g/L at 210 °C for 85.32 FPU of cellulose activity.

Table 1. Location of Relevant Indicator Bands in OPEFB Materials, the Assignment to Functional Group and Component

Wave Number Location	Vibration	Functional Group or Component	Component	References
1730	C=O	Aldehyde, ketone, carboxylic acids,	Hemicellulose	Kristensen *et al.* (2008)
		Ester linkage of carboxylic groups of ferulic acid and *p*-coumaric acids	Lignin	Zakaria *et al.* (2001)
1635	O-H	Adsorbed water	Hemicellulose	Smidt *et al.* (2005)
1600-1590	C=C	Aromatic skeleton	Lignin and Cellulose	Pandey (1999) Wetzel *et al.* (2001)
1246	C-O-C, C-O	Polysaccharides	Cellulose	
1232	Vc-o	Carboxyl group	Hemicellulose	Smidt *et al.* (2005)
1000-1200	C-O-C stretching C-O C-OH	Polysaccharides	Cellulose Lignin	Yang *et al.* (2007)

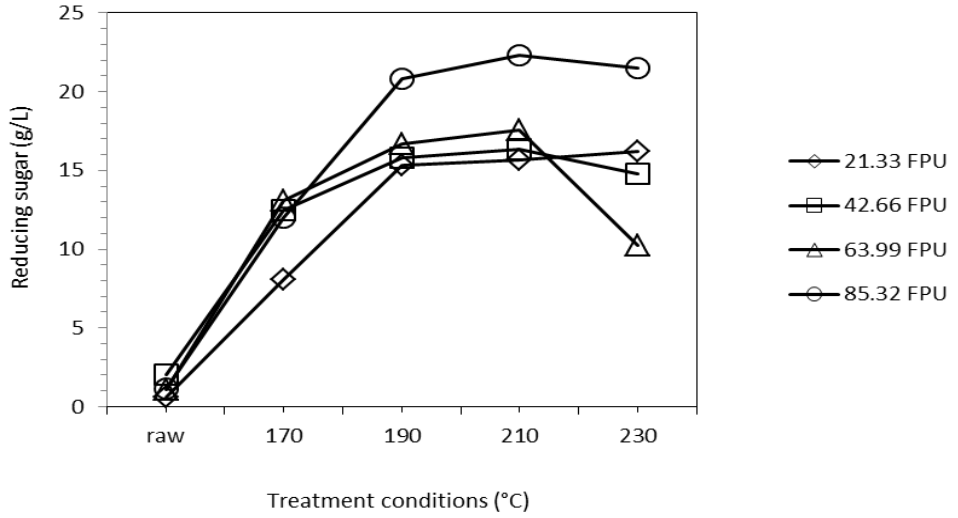

Fig. 4. Saccharification of treated OPEFB using different enzyme loading

Based on the results, the treated OPEFB gave 10 times higher sugar yield than the raw OPEFB. Shamsudin *et al.* (2012) reported that the heat produced from high-pressure steam treatment causes the moisture in the OPEFB to vaporize or expand and hydrolyzes part of the OPEFB component. The heat also caused the breakdown of gums and resins into soluble and insoluble oils, which loosened the fibrous structure of OPEFB, enhancing enzymatic hydrolysis. Conversely, as the treatment temperature was raised from 210 °C to 230 °C, the yield of reducing sugar decreased. This effect is probably due to the degradation of some of the cellulose component that could not be converted into glucose (Bahrin *et al.* 2012). These findings are supported by FTIR, SEM, and analysis of cellulose, hemicellulose, and lignin (CHL) results, which suggested that hemicellulose decomposition, silica body removal, and volatilization of some components occurred after the high-pressure steam treatment.

From the results, it was found that the reducing sugar yield was directly proportional to the enzyme concentration. As the enzyme concentration increased, the production of reducing sugar also increased. Several studies reported that the cost of raw material and enzymes is important in the bioconversion process of sugar. Hence, it is necessary to optimize enzymatic hydrolysis to reduce cost and at the same time obtain a high glucose yield. Therefore, this study suggested that high-pressure steam treatment at 210 °C and 2.03 MPa are optimum conditions to enhance the bioconversion of OPEFB into sugar.

Saccharification of Treated OPEFB at Different Temperatures

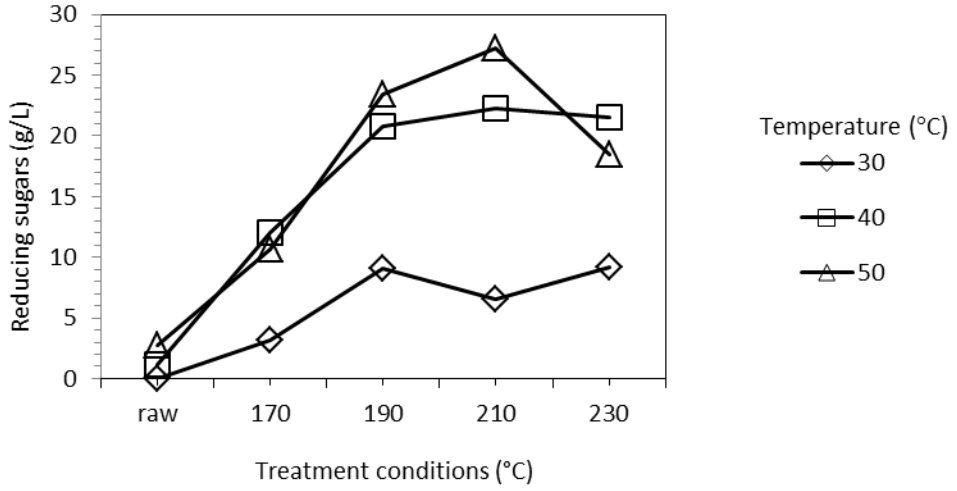

Fig. 5. Saccharification of raw and high-pressure steam treated OPEFB samples using different temperatures

The hydrolysis of untreated and treated OPEFB was performed at temperatures ranging from 30 °C to 50 °C (Fig. 5). As expected, untreated OPEFB resulted in the lowest reducing sugar for all hydrolysis temperatures within the experimental range. This could be attributed to the rigid structure of untreated OPEFB that does not allow the enzyme to penetrate. As a result, the cellulose component is not sufficiently accessible to enzymatic reaction.

The profiles of treated OPEFB samples indicate that the initial hydrolysis rate increases with an increase in temperature. Nevertheless, amongst the three hydrolysis temperatures, hydrolysis at 30 °C exhibited the lowest reducing sugar. According to Wu and Lee (1998), at 30 °C the enzyme appeared to have very limited impact on the reducing sugar yield since enzyme activity is very low. Raising the hydrolysis temperature from 40 °C to 50 °C helped obtain higher reducing sugar for OPEFB treated at 190 °C and 210 °C. Initially, the more heat that is added to the system, the faster the molecules move, thus increasing the number of collisions that provide sufficient energy to bring about the reaction.

From Fig. 5, the highest production of total reducing sugars was observed at the hydrolysis temperature of 50 °C for OPEFB treated at 210 °C, which resulted in 27 g/L of total reducing sugars. Meanwhile, when the treatment temperature was increased to 230 °C, the total reducing sugar for hydrolysis at 40 °C and 50 °C decreased to 21 g/L and 18 g/L, respectively. A slow decrease in reducing sugar might be due to the structural degradation of the cellulose component when treated at a high temperature. This result suggests that the best treatment condition for the hydrolysis of OPEFB was at 50 °C and the high-pressure steam treatment at 210 °C/2.03 MPa can significantly enhance the efficiency of the hydrolysis. This is in agreement with the findings of Vlasenko and Ding (1997), Krishna *et al.* (1998), and William *et al.* (2000) who also observed that a temperature of 50 °C was optimal for enzymatic hydrolysis of different lignocellulosic materials, such as rice straw, sugar cane, and corn stover.

Saccharification of High-Pressure Steam Treated OPEFB

Table 2 shows the results for enzymatic saccharification of the untreated and treated OPEFB at different temperatures of high-pressure steam. Increasing the treatment temperature from 170 °C to 210 °C caused the enzymatic hydrolysis yield of OPEFB to increase. Concurrently, the efficiency of enzymatic saccharification also improved and a higher glucose yield was achieved. Total reducing sugar was most favorable at 210 °C, whereby the highest hydrolysis percentage of 37.76% was attained. Based on this result, OPEFB treated at 210 °C also gave the maximum reducing sugar yield (17.57 g/L), compared to other temperatures.

The untreated OPEFB resulted in the lowest reducing sugar formation and also the lowest hydrolysis percentage of 2.79%. On the other hand, it was found that the hydrolysis yield of OPEFB treated at a temperature of 230 °C was reduced to 0.21 g/g, which corresponds to a hydrolysis percentage of 30.28%. This behavior might be due to the degradation of cellulose at high temperatures.

This result is agreement with the result of analysis of cellulose, hemicellulose and lignin (CHL) which found that the percentage ratio of cellulose and hemicellulose was reduced by 4.7% and 1.6%, respectively, when the treatment temperature was increased from 210 °C to 230 °C. This indicates that some of the cellulose and hemicellulose component was reduced during the treatment process. Therefore, it can be concluded that the optimum temperature for high-pressure steam treatment of OPEFB is 210 °C/2.03 MPa in order to achieve the highest conversion of bio-sugar.

Table 2. Saccharification of OPEFB at Different High-Pressure Steam Treatment Conditions

Temperature of OPEFB	Pressure (MPa)	Reducing Sugar (g/L)	Hydrolysis Percentage	Hydrolysis Yield (g/g)
Non-treated OPEFB	-	1.07	2.79	0.02
170 °C	0.82	13.06	33.75	0.26
190 °C	1.32	16.68	33.79	0.33
210 °C	2.03	17.57	37.76	0.35
230 °C	3.00	10.26	30.28	0.21

CONCLUSIONS

1. In this study, empty fruit bunches (OPEFB) were treated with high-pressure steam treatment (HPST) at different temperature and pressure conditions (170 ^{o}C/0.82 MPa), (190 ^{o}C/1.32 MPa), (210 ^{o}C/2.03 MPa), and (230 ^{o}C/3.00 MPa) for 2 min.
2. According to the results of CHL, FTIR, and SEM, it was found that HPST had successfully altered the lignocellulosic structure of OPEFB by degradation of hemicellulose and removal of silica bodies from the outer surface of OPEFB.
3. Results from this study demonstrated that 210 ^{o}C and 2.03 MPa were the optimal conditions of the HPST for enzymatic saccharification of OPEFB for which the highest hydrolysis percentage of 37.76% was attained. HPST is a promising method to improve the accessibility of the cellulose to enzyme and microbial attacks for the production of biosugar.

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REFERENCES CITED

Baharuddin, A. S., Abd Rahman, N. A., Md Shah, U. K., Hassan, M. A., Wakisaka, M., and Shirai, Y. (2011). "Evaluation of press shredded empty fruit bunch (EFB)-palm oil mill effluent (POME) anaerobic sludge based compost using Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis," *Afr. J. Biotechnol.* 10(41), 8082-8089.

Baharuddin, A. S., Lim, S. H., Md. Yusof, M. Z., Abdul Rahman, N. A., Md. Shah., U. K., Hassan, M. A., Wakisaka, M., Sakai, K., and Shirai, Y. (2010). "Effects of palm

oil mill effluent (POME) anaerobic sludge from 500 m^3 of closed anaerobic methane digested tank on press-shredded empty fruit bunch (EFB) composting process," *Afr. J. Biotechnol.* 9(16), 2427-2436.

Bahrin, E. K., Baharuddin, A. S., Ibrahim, M. F., Abdul Razak, M. N., Sulaiman, A., Abd-Aziz, S., Hassan, M. A., Shirai, Y., and Nishida, H. (2012). "Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam," *BioResources* 7(2), 1784-1801.

Balan, V., Sousa, L. D., Chundawat, S. P. S., Vismeh, R., Jones, A. D., and Dale, B. E. (2008). "Mushroom spent straw: A potential substrate for an ethanol-based biorefinery," *J. Ind. Microbiol. Biot.* 35(5), 293-301.

Chang, V. S., and Holtzapple, M. T. (2000). "Fundamental factors affecting biomass enzymatic reactivity," *Applied Biochem. Biotechnol*. 84, 5-37.

Goering, H. K., and Van Soest, P. J. (1970). "Forage fiber analysis," *Agricultural Handbook No. 379*, US Department of Agriculture, Washington, DC, 1-20.

Gupta, R. B., and Demirbas. A. (2010). "Gasoline, diesel and ethanol biofuel from grasses and plants," *Ethanol from Biomass by Fermentation*, Cambridge University Press, USA, 84-101.

Hari Krishna, S., Prasanthi, K., Chowdary, G. V., and Ayyanna, C. (1998). "Pretreated sugarcane leaves to ethanol," *Process Biochemistry* 33(8), 825-830.

Hendriks, A. T. W. M., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol*. 100, 10-18.

Hubbe, M. A., Nazhad, M., and Sánchez, C. (2010). "Composting as a way to convert cellulosic biomass and organic waste into high-value soil amendments: A review," *BioResources* 5(4), 2808-2854.

Kristensen, J. B., Thygesen, G. L., Felby, C., Jorgensen, H., and Elder, T. (2008). "Cell wall structural changes in wheat straw pretreated for bioethanol production," *Biotechnol. Biofuels* 1-5.

Latif, F., Ibrahim, M. R., and Kauser, A. M. (1994). "Saccharification of *Leptochoa fusca* (Kallar grass straw) using thermostable cellulases," *Bioresour. Technol*. 50(2), 107-111.

Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal Jr., M. J., and Lynd, L. R. (2002). "A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol," *Bioresour. Technol*. 81, 33-44.

Laureano-Perez, L., Teymouri, L., Alizadeh, H., and Dale, B. E. (2005). "Understanding factors that limit enzymatic hydrolysis of biomass," *Appl. Biochem. Biotechnol.*, 1081-1099.

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem*. 31(3), 426-428.

Neethirajan, S., Gordon, R., and Wang, L. (2009). "Potential of silica bodies (phytoliths) for nanotechnology," *Trends Biotechnol*. 27(8), 461-467.

Palmowski, L., and Muller, J. (1999). "Influence of the size reduction of organic waste on The anaerobic digestion," *II International Symposium on Anaerobic Digestion of Solid Waste*, Barcelona, 15-17 June, 137-144.

Pandey, K. K. (1999). "A study of chemical structure of soft and hardwood and wood polymers by FTIR spectroscopy," *J. Appl. Polym. Sci*. 71, 1969-1975.

Ramos, L. P. (2003). "The chemistry involved in the steam treatment of lignocellulosic materials," *Quim Nova* 26, 863-871.

Singh, S., Simmons, B. A., and Vogel, K. P. (2009). "Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass," *Biotechnology and Bioengineering* 104, 68-75.

Shamsudin, S., Md Shah, U. K., Zainudin, H., Abd-Aziz, S., Mustapa Kamal, S. M., Shirai, Y., and Hassan, M. A. (2012). "Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars," *Biomass and Bioenergy* 36, 280-288.

Shuit, S. H., Tan, K. T., Lee, K. T., and Kamaruddin, A. H. (2009). "Oil palm biomass as a sustainable energy source: A Malaysian case study," *Energy* 34(9), 1225-1235.

Smidt, E., and Schwanninger, M. (2005). "Characterization of waste materials using FTIR spectroscopy: Process monitoring and quality assessment," *Spectrosc. Lett.* 38, 247-70.

Sun, Y., and Cheng, J. Y. (2002). "Hydrolysis of lignocellulosic materials for ethanol production, A review," *Bioresource Technology* 83(1), 1-11.

Taniguchi, M., Suzuki, H., Watanabe, D., Sakai, K., Hoshino, K., and Tanaka, T. (2005). "Evaluation of pretreatment with *Pleurotus ostreatus* for enzymatic hydrolysis of rice straw," *J. Biosci. Bioeng.* 100(6), 637-643.

Vlasenko, E. Y., and Ding, H. (1997). "Enzymatic hydrolysis of pretreated rice straw," *Bioresource Technology* 59(19), 109-119.

Wetzel, D. L. (2001). "Fundamental processes within natural and constructed wetland ecosystems: Short-term versus long-term objectives," *Proceedings of the International Wheat Quality Conference II*, May 2001, Manhattan, Kansas, USA, 1-20.

Wu, Z., and Lee, Y. Y. (1998). "Nonisothermal simultaneous saccharification and fermentation for direct conversion of lignocellulosic biomass to ethanol," *Applied Biochemistry and Biotechnology* 70-72(1), 479-492.

Yang, H., Yan, R., Chen, H., Lee, D. H., and Zheng C. (2007). "Characteristics of degraded cellulose," *Polym. Degrad. Stab*. 94(9), 1379-1388.

Zabihi, S., Alinia, R., Esmaeilzadeh, F., and Kalajahi, J. F. (2010). "Pre-treatment of wheat straw using steam, steam/acetic acid and steam/ethanol and its enzymatic hydrolysis for sugar production," *Bioresource Technology* 105, 288-297.

Zakaria, S., Hamzah, H., Murshidi, J. A., and Deraman, M. (2001). "Chemical modification on lignocellulosic polymeric oil palm empty fruit bunch for advance material," *Adv. Polym. Technol.* 20, 289-295.

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