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Der Pharma Chemica, 2014, 6(5):1-6
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Effect of direct pretreatment using steam on the properties of oil palm empty fruit bunch

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ABSTRACT

The complex structure and existence of lignin hinder the hydrolysis of cellulose and hemicellulose in lignocellulosic biomass. This study was carried out to determine the properties of oil palm empty fruit bunch directly treated with steam without size reduction and acid/alkali treatments. The temperature and time of pre-treatment was varied and found that there was no significant difference in the production of glucose and xylose after Cellic CTec2 enzymatic hydrolysis. Furthermore, pre-treatment neither changed in the moisture content nor the pH values in oil palm empty fruit bunch; however, a small reduction in pH was observed due to the generation of acetic acid, formic acid and levulinic acid. Thus, pre-treatment of the samples yields higher cellulose and lesser lignin content.

Keywords: oil palm empty fruit bunch; lignocellulose; pre-treatment; hydrolysis; Cellic CTec2

INTRODUCTION

Palm oil is one of the most economical oil crops cultivated mainly in Malaysia and Indonesia. Malaysia is the second largest palm oil producer in the world [1]. High production of palm oil generates large amount of solid wastes, namely oil palm empty fruit bunch (OPFB), oil palm mesocarp and oil palm shell. Almost 15 million tons of this agriculture waste is generated by oil palm industries annually which pose enormous environmental pollution [2,3].

Lignocellulosic biomass is a renewable source that stores energy from sunlight into chemical bonds [4]. According to International Energy Agency (IEA), biomass supplies account for 14% of total world's energy requirement. Therefore, it plays a key role in enhancing the economic welfare of the country [5]. OPFB is an ideal low-cost feedstock used in the production of fuel ethanol through pretreatment, hydrolysis and fermentation [1]. It comprises nearly 42-65% cellulose, 17-34% hemicellulose, 13-25% lignin, 1-6 % ash and 63-67% moisture [6-8]. Cellulose and hemicellulose composed of several units of carbohydrate monomers which are hydrolyzed by enzymes into sugars while lignin is an amorphous hetero-polymer made up of phenylpropane units [9]. The effectiveness of hydrolysis depends mainly on the accessibility of enzyme to the substrate. Thus, any barrier in the accessibility of enzyme to the lignocellulose reduce hydrolysis rate significantly.

The preferred method used for the degradation of cellulose is a heat and/or chemical pretreatment followed by enzymatic hydrolysis [10]. Effective pretreatment is an important step in the success of lignocellulosic bioconversion where polymer sugars from cellulose and hemicellulose are hydrolyzed into free monomer. These

monomeric sugars further used in fermentation to produce bioethanol. Pretreatment breaks lignin seal, reduces crystallinity of cellulose thus increase porosity which make cellulose more accessible to the enzymes. Furthermore, it increase conversion of carbohydrates into fermentable sugars decreasing the overall process cost [11]. In general procedure, OPFB is firstly subjected to size reduction steps prior to steam pretreatment to increase the surface area. Pretreatment usually involves the use of acids and alkalis. Although, the use of chemicals is tolerable at laboratory scale, it may generate environmental problems when used at industrial scale. Thus this study was conducted to determine the effect of steam on OPFB in its available form without use of chemicals during pretreatment.

In the present study, mixture of cellulase and hemicelulase called Cellic CTec2 enzyme was used for bioconversion of OPFB. Samples were firstly pretreated with steam at different temperatures for different time intervals followed by their enzymatic hydrolysis. The produced sugars were analyzed by high performance liquid chromatography (HPLC). The fiber composition of treated OPFB samples was studied using National Renewable Energy Laboratory (NREL) as reference.

MATERIALS AND METHODS

Chemicals

Enzyme Cellic CTec2, a mixture of cellulase and hemicellulase was obtained from Novozyme. Sigmacell Type 20 cellulose, 3,5-dinitrosalicylic (DNS), ethanol, glucose and xylose were purchased from Sigma Aldrich Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and used without further purification.

Raw material

The OPFB was obtained from Sime Darby Sdn Bhd, Selangor, Malaysia. Samples were packed in polyamide nylon plastic bag and stored at -20 °C to prevent any microbial growth. Before pretreatment, samples were defrosted for 1 hr at ambient temperature, washed several times with distilled water to remove any unnecessary foreign material and dried overnight at 105°C. Change in weight and percent moisture content was calculated. Total mass was calculated by deducting moisture content from 100%. The dried OPFB was grounded into approximately 0.5 mm particle size using a mill (Fritsch GmBH, Germany).

Steam pre-treatment

Steam pre-treatment of OPFB was carried out in an autoclave at 110 °C and 130 °C for 20, 40 and 60 minutes. The treatment was done with heated steam without adding any acid or alkali using randomized experimental design.

Determination of enzyme activity

Cellic CTec2 activity was determined according the procedure described by Adney and Baker with some modifications [12]. The unit of enzyme activity was calculated in BHU (Biomass Hydrolysis Unit) which refers to the amount of reducing sugar produced by 1 mL of enzyme reacting with 1 gram of biomass per minute. In the present study, substrates used were OPFB fiber while Sigma Cell Type 20 cellulose was taken as a control. Five different enzyme solutions from stock were prepared in buffer to the ratio of 1:20. Assay solutions contained 0.01 g of OPFB with varying amounts of 0.05M pH 4.8 sodium citrate buffer and 0.5 mL of Cellic CTec2 enzyme. Hydrolysis was carried out at 50°C for 60 minutes and reducing sugar produced was determined spectrophotometrically using 3,5-dinitrosalicylic acid. A linear graph of reducing sugar was plotted against enzyme concentration and Cellic CTec2 activity was determined using the following equation

$$\text{Biomass hydrolysis unit (BHU)} = \frac{\text{Slope of linear line}}{\text{Substrate weight (gm) x enzyme volume (mL) x time (min)}}$$

Enzymatic saccharification of OPFB

Saccharification of OPFB was carried out by Cellic CTec2 according to the procedure described by Saleha et al [13]. It was performed using 5% (w/v) substrate and 0.05% (v/v) enzyme in a flask. Reaction mixture was placed in a shaker at 150 rpm for 72 hours with maintained 50°C. After hydrolysis, 3.0 mL hydrolysate was filtered with a nylon membrane (0.45µm pore size) and sugar was analyzed by HPLC equipped with evaporative light scattering detector (ELSD) (Waters, Milford, USA). Purospher STAR NH_2 HPLC column with 5 mm particle size was used and eluted

with HPLC grade acetonitrile and deionized water in a ratio of 80:20. Glucose and xylose were used as standard sugars for qualitative and quantitative analysis.

Analysis of chemical composition

Chemical composition of OPFB samples were analyzed using modified National Renewable Energy Laboratory (NREL) standard biomass analytical procedure. Moisture content was analyzed according to standard AOAC method (1984).

Analysis of moisture content

For the determination of moisture content a disk was heated at 105 ^{o}C for one hour followed by its cooling in a dessicator. OPFB (0.30±0.01g) was weighed and heated witin disk at 105 ^{o}C for 24 hours. The disk containing sample was weighed and moisture content was calculated.

Analysis of ash content

Ash content was determined according to the method of Sluiter et al [14]. A crucible was first heated at 575±25°C for 4 hours and cooled in a dessicator. OPFB (1.5±0.1g) was placed into the crucible and heated at 500 °C until no smoke was seen. The sample was further heated at 575±25 °C for 24 hours, cooled in the dessicator and weighed. The ash content was determined using the following equation

$$\text{Amount of Ash (\%)} = \frac{\text{Initial crucible weight (g)} - \text{final crucible weight (g)}}{\text{Sample weight (g)}}$$

Determination of total extractives

Total extractives were calculated according to the procedure described by Sluiter et al [15]. In short, 3.0±0.1 g OPEB sample was kept in 95x70 mm tea bag and placed in a cellulose thimble. The extraction was caried out for 24 hours in 500 mL round-bottom flask having 200±10 mL distilled water. After water extraction, soxhlet system cooled and replaced with 95% (v/v) ethanol. The reflux process was performed for 7 hours. After extraction, samples were removed and dried in an oven at 40^{o}C for 8 hours. The weight of the sample was calculated and moisture content was determined as described previously.

Total lignin content

Total lignin content was determined according to the method described by Sluiter et al [16]. OPFB fiber (0.30±0.01g) was placed into 100 mL cone cylinder having 3.0±0.1mL of 72% (v/v) sulphuric acid. Hydrolysis was carried out in a shaker at 150 rpm for 90 minutes (30 ^{o}C). After hydrolysis, distilled water was added to dilute sulphuric acid upto 4% (v/v). Again hydrolyzed at 121 ^{o}C for 1 hour in an autoclave and filtered. The residue left on the filter paper was dried in an oven for overnight at 60 ^{o}C. Final weight of the remains after acid hydrolysis was assumed as acid insoluble lignin while its filtrate was taken as acid soluble lignin. Acid soluble lignin was determined spectrophotometrically at 250 nm. The total lignin was the sum of acid soluble and acid insoluble lignin and determined using the following equations

$$\text{Acid insoluble lignin (\%)} = \frac{\text{Weight of residue (g)} - \text{weight of filter paper (g)} - \text{weight of ash (g)} \times 100}{\text{Weight of sample (g)}}$$

$$\text{Acid Soluble lignin (\%)} = \frac{(A/ab) \times df \times V \times 100}{W}$$

Where A= absorption at the wavelength 205nm; *df* = dilution factor; b = cell path length (1cm); a= absorption(110L/g-cm); V= volume of filtration (0.087L); W= initial sample weight in grams

Statistical analysis
ANOVA and DUNCAN's tests were carried out using the software Statistical Analysis System (SAS) Version 9.1. $p<0.05$ was selected as the level of statistically significant.

RESULTS AND DISCUSSION

Determination of Celic CTec2 activity
Cellic CTec2 is a commercial enzyme consists of cellulase, β-glucosidase and hemicellulase activity. It has been reported that the β-glucosidase increase the efficiency of enzyme system because it remove cellulobiose that retards enzymatic hydrolysis [17]. The enzyme activity of Cellic CTec2 was 4379.33 and 2527.27 BHU for Sigma cell Type 20 and OPFB substrate respectively. The enzyme activity of the control was found higher as compared to OPFB because of the Sigma cell Type 20 cellulose taken as the control was pure in powdered form.

Moisture content
Table 1 shows the change of moisture content in OPFB before and after steam pre-treatment. The results illustrated that there is no significant difference in moisture content after pretreatment. The small increment after pretreatment is related to the hydroxyl group of cellulose and lignin which absorb water easily through hydrogen bonds [18].

Table 1. Moisture content in OPFB before and after pre-treatment

Treatment	Moisture content before pre-treatment (%)	Moisture Content after pre-treatment (%)
Untreated	58.63 ± 5.25^{ab}	-
110 °C, 20 min	55.08 ± 4.53^{b}	60.53 ± 3.44^{a}
110 °C, 40 min	57.36 ± 3.31^{ab}	62.13 ± 3.12^{a}
110 °C, 60 min	58.59 ± 6.93^{ab}	60.04 ± 5.16^{a}
130 °C, 20 min	58.63 ± 3.48^{ab}	61.47 ± 2.12^{a}
130 °C, 40 min	62.64 ± 3.96^{a}	62.76 ± 5.41^{a}
130 °C, 60 min	60.90 ± 1.93^{ab}	60.58 ± 3.40^{a}

** a-b*
Different alphabets indicate significant differences ($p<0.05$) between both samples.

Ash, total extractive and solid mass
Ash content is the residue remaining after dry oxidation at 575 °C. It consists of mineral and other non-organic material bound to the physical structure of biomass. The total extractive content is an extractable biomass that contains water and ethanol soluble material. It includes non-structural biomass components which could disrupt the effectiveness of hydrolysis [14,15]. The percentage of ash and total extractive content in OPFB was 3.01% and 28.83 %, respectively (Table 2).

The mass of raw OPFB was significantly different from washed sample (Table 2). Washing eliminated water soluble components and foreign material. Raw sample exhibited higher solid content compared to washed OPFB since raw sample has higher amount of foreign material that interfere in direct heating and complete degradation of lignin [19].

Table 2. Ash, total extractable and solid mass in OPFB extract

Component	Percentage content (%)
Ash*	3.01 ± 1.11
Total extractable*	28.83 ± 13.44
Solid content in raw OPEFB fiber*	58.55 ± 0.36
Solid content after washing*	31.63 ± 1.96

**Percentage in wet basis*

Lignin content after steam pre-treatment
Lignin content in OPFB was determined by acid hydrolysis (Table 3). At 110 °C, increasing the heating time from 20 to 40 min did not show any significant differences but increasing the heating time further to 60 min resulted in a significantly lower ($p<0.05$) lignin content. However, at a steam temperature of 130 °C, an increase of heating time from 20 to 60 min did not affect the lignin content.

It was interesting to observe that a heating duration of 20 min although increased the temperature from 110 °C to 130 °C yet it did not affect any significant difference in lignin content. However, when heating time was further increased to 40 min increasing the temperature to 130 °C caused the lignin content to be significantly lower

($p<0.05$). Increasing the temperature to 130 °C during heating for 60 minutes did not significantly affect lignin content.

Lignin content decreased at certain heating time and temperature because auto-hydrolysis promotes removal of hemicelluloses and degradation or modification of lignin [13]. Steam treatment at high temperature and pressure expanded existing moisture and increased the hydrolysis of OPFB because it unwrap biomass particle structure leading to increased fiber volume and reduction of particle size [20].

Table 3. Lignin content in OPEFB after pre-treated at different temperatures and time

Time (minutes)	Temperature (°C)	Temperature (°C)
	110	130
20	35.52 ± 3.99 [a]	30.67 ± 13.51[ab]
40	34.47 ± 2.81[a]	21.15 ± 11.92[b]
60	20.96 ± 6.03[b]	25.92 ± 4.79[ab]
No treatment	34.69 ± 4.91[a]	

a-b different alphabets represent significant difference ($p<0.05$) between samples

Effect of temperature and pretreatment time on the production of sugars

The effect of pre-treatment on the production of sugar in OPFB is shown in Table 4. The result demonstrated that there was no significant difference ($p>0.05$) in the formation of glucose and xylose in untreated and steam pretreated samples. Both the temperature and time have insignificant effects on sugar production. According to Saleha et al the pre-treatment with steam at 140°C for 15 min increased the conversion rate of holocellulose in the oil palm empty fruit bunch by 30% [13]. The inefficiency of pretreatment may be due to low surface to volume ratio of OPFB as it was not milled into smaller particles.

Table 4. Glucose and Xylose in OPFB for wet and dry basis after enzyme hydrolysis

Treatment	Wet basis		Dry basis	
	Glucose (mg/g)	Xylose (mg/g)	Glucose (mg/g)	Xylose (mg/g)
Untreated	68.82 ± 65.34[a]	31.32 ± 28.89[a]	165.90 ± 148.01[a]	73.49 ± 63.33[a]
110 °C, 20 min	53.23 ± 34.99[a]	42.98 ± 24.84[a]	132.58 ± 82.62[a]	105.98 ± 54.61[a]
110 °C, 40 min	33.77 ± 42.82[a]	39.04 ± 19.23[a]	90.15 ± 113.90[a]	101.85 ± 46.85[a]
110 °C, 60 min	77.06 ± 26.21[a]	44.99 ± 23.06[a]	193.76 ± 72.82[a]	112.46 ± 58.31[a]
130 °C, 20 min	62.42 ± 67.67[a]	36.87 ± 29.80[a]	158.14 ± 163.57[a]	93.25 ± 71.86[a]
130 °C, 40 min	76.12 ± 70.94[a]	50.06 ± 47.54[a]	193.26 ± 172.15[a]	124.94 ± 110.01[a]
130 °C, 60 min	103.49 ± 93.46[a]	55.15 ± 38.91[a]	252.74 ± 214.50[a]	135.60 ± 88.87[a]

[a] *Means with the same letter showed no specific difference*

Effect of hydrolysis and pre-treatment on pH values of OPFB

Change in pH before and after hydrolysis of OPFB is shown in Table 5. The result revealed that the pH was nearly same in the untreated and samples treated with steam. A small decrease in pH might be due to the release of acid. Pre-treatment with steam is an auto hydrolysis and catalyze the release of acids leading to the lowering pH. It generates acetic acid, formic acid and levulinic acid [21]. The pH after enzymatic hydrolysis was significantly lower ($p<0.05$) in case of untreated samples. However, among treated samples no remarkable changes were observed.

Table 5. Changes in sample pH before and after enzymatic saccharification for 72 hours

Treatment	Sample pH before enzymatic hydrolysis	Sample pH after enzymatic hydrolysis
Untreated	5.61 ± 0.79[a]	3.90 ± 0.05[b]
110 °C, 20 min	5.42 ± 0.45[ab]	4.37 ± 0.08[a]
110 °C, 40 min	5.33 ± 0.38[ab]	4.47 ± 0.28[a]
110 °C, 60 min	5.38 ± 0.21[ab]	4.47 ± 0.27[a]
130 °C, 20 min	5.13 ± 0.18[ab]	4.45 ± 0.12[a]
130 °C, 40 min	5.02 ± 0.12[b]	4.20 ± 0.05[a]
130 °C, 60 min	4.93 ± 0.07[b]	4.30 ± 0.24[a]

a-b different alphabets represent significant difference ($p<0.05$) between samples.

CONCLUSION

This study shows that the steam pretreatment of OPFB in its available form does not significantly improve sugar yield without requirement of any size reduction step and the usage of any acid or alkali. Furthermore, lignin content considerably reduces at 130 °C temperature with heating time of 40 min.

Acknowledgements
The authors gratefully acknowledge to Universiti Kabangsaan Malaysia for the research grant DPP-r013-026, that has made this work possible. The authors wish to thank to Sime-darby, Banting for providing experimental materials.

REFERENCES

[1] S. Sumathi, S.P. Chai, A.R. Mohamed, *Renewable and Sustainable Energy Reviews* **2008,** 12, 2404.
[2] K.N. Law, W.R.W. Daud, A. Ghazali, *BioResources* **2007**, 2, 351.
[3] M. Suhaimi, *Teknologi Makanan, MARDI* **1990**, 9, 57.
[4] P.McKendry, *Bioresource Technology* **2002**, 83, 37.
[5] A. Yousuf, *Waste Management* **2012**, 32, 2061.
[6] D. Piarpuzán, J.A. Quintero, C.A. Cardona, Biomass *and Bioenergy* **2011,** 35, 1130.
[7] S. Shinoj, R. Visvanathan, S. Panigrahi, M. Kochubabu, *Industrial Crops and Products* **2011**, 33, 7.
[8] A. Verardi, I. De Bari, E. Ricca, V. Calabro, *Bioethanol, Marco Aurelio Pinheiro Lima* (Ed.), **2012**, 978.
[9] L.R. Lynd, P.J. Weimer, W.H. Van Zyl, I.S. *Microbiology and molecular biology reviews* **2002,** 66**,** 506.
[10] K.A. Gray, L. Zhao, M. Emptage, *Current Opinion in Chemical Biology* **2006**, 10**,** 14.
[11] Y. Yamashita, C. Sasaki, Y. Nakamura, *Journal of Bioscience and Bioengineering* **2010**, 110**,** 79.
[12] B. Adney, J. Baker, *Laboratory Analytical Procedure* Colorado: National Renewable Energy Laboratory. **1996**, 6.
[13] S. Saleha, M.S. Umi Kalsom, Z. Huzairi, A.A. Suraini, M.K. Siti Mazlina, Y. Shirai, H. Mohd Ali, *Biomass and Bioenergy* **2012**, 36, 280.
[14] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, *NREL Laboratory Analytical Procedure (LAP)*, **2005a**.
[15] A. Sluiter, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, *NREL Laboratory Analytical Procedure*, **2005b**.
[16] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, *NREL Laboratory Analytical Procedure (LAP)*, **2008**.
[17] L.F. Martins, D. Kolling, M. Camassola, A.J.P. Dillon, L.P. Ramos, *Bioresource Technology* **2008,** 99**,** 1417.
[18] M. Karina, H. Onggo, A.H.D. Abdullah, A. Syampurwadi, *Journal of Biological Sciences* **2008**, 8**,** 101.
[19] N. Abdullah, F. Suleiman, H. Gerhauser, *Journal of Physical Science* **2011**, 22, 1.
[20] G. Michalowicz, B. Toussaint, M.R. Vignon, *Holzforschung* **1991,** 45, 175.
[21] L.P. Ramos, *Química Nova* **2003**, 26, 863.