**Combined pretreatment with hot compressed water and wet disk
milling opened up oil palm biomass structure resulting in enhanced
enzymatic digestibility**

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ABSTRACT

Combined pretreatment with hot compressed water and wet disk milling was performed with the aim to reduce the natural recalcitrance of oil palm biomass by opening its structure and provide maximal access to cellulase attack. Oil palm empty fruit bunch and oil palm frond fiber were first hydrothermally pretreated at 150°C to 190°C and 10 to 240 min. Further treatment with wet disk milling resulted in nanofibrillation of fiber which caused the loosening of the tight biomass structure, thus increasing the subsequent enzymatic conversion of cellulose to glucose. The effectiveness of the combined pretreatments were evaluated by chemical composition changes, power consumption, morphological alterations by SEM and the enzymatic digestibility of treated samples. At optimal pretreatment process, approximately 88.5% and 100.0% of total sugar yields were obtained from oil palm empty fruit bunch and oil palm frond fiber samples, which only consumed about 15.1 and 23.5 MJ/kg of biomass, respectively.

Keywords: Oil palm empty fruit bunch, oil palm frond fiber, hot compressed water, wet disk milling, combined pretreatment.

1. Introduction

Oil palm biomass is a renewable resource, cheap and available throughout the year, and not properly-utilized with lower impact value (Lee and Ofori-Boateng, 2013). Therefore, appropriate technologies for biomass upgrading is sought to increase the value and to promote sustainable growth of the oil palm industry. Lignocellulosic biomass is an importance source of natural biopolymers on the earth and their rigid and intact structures of cellulose-hemicellulose-lignin complexes are linked with various types of chemical bonds which protect them from chemical and biological degradation (Mosier et al., 2005). A number of technologies have been reported in the literature to fractionate lignocellulosic biomass and the development of chemical-free approaches with environmentally-sound technology. Furthermore low energy consumption with consideration of maximum recovery of pentose and hexose sugars are currently in progress (Hideno et al., 2009; Lee et al., 2010; Kumagai et al., 2014).

Oil palm empty fruit bunch (OPEFB) and oil palm frond fiber (OPFF) have been treated by various methods such as mechanical, chemical and thermochemical pretreatment process (Palamae et al., 2014; Cheng et al., 2014; Ofori-Boateng and Lee, 2014; Sabiha-Hanim et al., 2011). Previous ball milling pretreatment process demonstrated high energy usage with high recovery of both pentose and hexose sugars (Zakaria et al., 2014a). Hydrothermal pretreatment alone achieved high conversion of

cellulose to glucose at higher pretreatment temperature with **loss of pentose sugars by**
conversion to furfural and formic acids (Zakaria et al., 2015a). Hydrothermal
pretreatment with subsequent wet disk milling (WDM) exhibited excellent recovery of
pentose and hexose sugars with lower cellulase loadings (Lee et al., 2010; Hiden et al.,
2012; Weiqi et al., 2013; Kumagai et al., 2015). Superheated steam (SHS) treatment was
tested on oil palm mesocarp fiber (OPMF) and the glucose conversion yield was not
pronounced in comparison to hot compressed water (HCW) due to minimal contact of
water that reacts as catalyst in the hydrolysis of hemicellulose component. Combination
of pretreatment using HCW (180°C for 20 min) and WDM were the best method to
recover the highest xylose (90%) and glucose (86%), and was preferred for pretreatment
of OPMF (Zakaria et al., 2015b).

Therefore in this study, individual pretreatment of HCW, WDM and
combination of them were conducted to understand and improve the conversion of
hemicellulose and cellulose from OPEFB and OPFF samples. HCW was performed
under isothermal and non-isothermal conditions to compare the behaviour of the
hemicellulose degradation by-products generated from pretreated liquids and properties
of reactive cellulose from pretreated solids. Energy requirement by WDM from different
pretreatment severities were thoroughly compared and discussed.

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84   **2.   Materials and Methods**

85   *2.1. Hot compressed water (HCW) pretreatment*

86   The OPEFB was collected from Seri Ulu Langat Palm Oil Mill, Dengkil,
87   Selangor, Malaysia, and the OPFF was obtained from the oil palm plantation at
88   Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The samples were prepared as
89   discussed earlier ([Zakaria et al., 2014a](#)) prior to component analysis. Hot compressed
90   water (HCW) pretreatment was performed using a 1 L stainless steel autoclave (Nitto
91   Koatsu Co., Tsukuba, Japan) ([Zakaria et al., 2015b](#)). The oil palm biomass was
92   pretreated at temperature ranges from 150°C to 190°C for 10 min and 240 min,
93   respectively. The pretreated solids were separated from the slurry by using Advantec No.
94   2 membrane filter and rinsed with distilled water until neutral pH. **The soluble sugars in**
95   **pretreated liquids were identified by HPLC as describing below.** The pretreated solid
96   portion recovered after filtration were subjected to subsequent treatment using wet disk
97   milling. The intensity of the hydrothermal treatment was expressed as severity factor
98   (log Ro). The severity parameters corresponding to different hydrothermal pretreatment
99   conditions are calculated as in Eq. (1), in which t is the reaction time (min), and T is the
100   hydrolysis temperature (°C) ([Overend and Chornet, 1989](#)).

$$R_o = t_{exp} [(T-100)/14.75] \quad (1)$$

2.2. Wet disk milling (WDM) pretreatment

Wet disk milling was performed using Supermass colloidizer MKZA10 (Masuko Saangyo Co., Ltd., Saitama, Japan) following the method reported earlier (Zakaria et al., 2015b). The pretreated samples were centrifuged at 10,000 x g for 15 min and the solids recovered were subjected to freeze-drying prior to enzymatic hydrolysis and other analysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed using an enzyme cocktail constituting 40 FPU/mL *Acremonium* cellulase (Meiji Seika Co, Japan), and 10% Optimash BG (Genencor International, California, USA) as reported previously (Zakaria et al., 2014b). The enzymatic hydrolysis was performed at 50°C for 72 h with stirring/shaking. The experiment was performed in triplicates and the results are presented as the average values. The enzymatic digestibility was evaluated by the obtained sugars (mg sugars/g materials) or sugar yield as calculated using Eq. (2):

Sugar yield (%) = [weight of monomeric sugars after enzymatic hydrolysis/weight of total monomeric sugars of biomass after hydrolysis using H₂SO₄] × 100 (2)

2.4. Energy consumption in WDM pretreatment

The actual measurement values of power consumption during WDM were used for energy calculation as reported earlier ([Hideno et al., 2009](#)).

2.5. Analytical methods

Detection of monomeric and oligomeric sugars, acetic acid, furfural, 5-HMF, and formic acid from untreated materials, HCW-treated and enzymatic hydrolysis were performed using high-performance liquid chromatography (HPLC) according to the analytical method (NREL/TP-510-42623) ([Sluiter et al., 2008](#)). Morphological analysis was conducted by using scanning electron microscope (SEM; S-3400 and S-4800, Hitachi, Japan) at 1.0 kV. The preparation of fibrillated pretreated samples was performed as reported earlier ([Zakaria et al., 2015b](#)).

3.0 Results and Discussion

3.1 Effect of pretreatment severities on pretreated liquids

Previous works reported hydrothermal pretreatment of oil palm biomass under non-isothermal conditions with short reaction time (Zakaria et al., 2015a). In contrast, in the present study oil palm biomass is hydrothermally pretreated under both isothermal and non-isothermal conditions with mild reaction temperature and longer reaction time to understand the degradation mechanism of hemicellulose and degradation by-products formation as well as reactive cellulose recovered. Formation of hydrolyzed sugars from OPEFB and OPFF samples are presented in Table S1. In general, both monomeric and oligomeric sugars were increased with higher pretreatment severities (either higher temperature or longer reaction time). The highest monomeric and oligomeric sugars detected were xylose, followed by glucose, arabinose, galactose and mannose. The highest xylo-oligomers (XOS) detected was 4.8 g/ 100 g of OPEFB samples at 190°C, 20 min (log Ro = 4.0) (Table S1). This value is equivalent to 30.8% of XOS from OPEFB samples. Meanwhile, XOS generated from OPFF was 4.5 g/ 100 g of OPFF at 190°C, 10 min (log Ro = 3.6) which is about 26.6% from OPFF sample. It was obvious that the production of XOS was greater at the higher temperature than reaction time. This is because hemicellulose degradation takes place at or greater than 180°C (Bobleter, 1994). The monomeric and oligomeric glucose, arabinose, galactose and mannose levels were

155 higher from OPFF samples compared to OPEFB probably due to the differences in
156 hemicellulose components, amounts and molecular structures of the fibers. A lower XOS
157 production from OPEFB recorded in this study compared to study reported by [Ho et al.](#)
158 (2014) probably due to treatment severity applied and a ratio of solid to liquid used.

159 Acetic acid, furfural, 5-hydroxymethylfurfural (5-HMF) and formic acid were
160 among the degradation by-products detected in the pretreated liquids and the
161 concentrations of each product at different pretreatment severities are presented in [Fig.](#)
162 [S1](#). Acetic acids were observed in increasing trend towards higher pretreatment severities
163 and reached the highest at 13.2 mg/g of OPEFB and 12.6 mg/g of OPFF at 190°C, 20 min
164 and 190°C, 10 min, respectively. Acetic acids accumulation occurring at higher
165 pretreatment severities resulted from deacetylation of hemicellulose degradation ([Esteves](#)
166 [and Pereira, 2009](#)). Different furfural and 5-HMF profiles are observed from OPEFB and
167 OPFF, respectively. Furfural was only detected at pretreatment condition of 150°C, 240
168 min and 190°C, 20 min with concentration of 0.6 mg/g and 2.4 mg/g, respectively from
169 OPEFB samples. Meanwhile furfural formation was not generated when OPFF was
170 pretreated between 150°C-170°C for 10 min. 5-HMF was not detected from OPEFB
171 samples at all pretreatment conditions tested. In contrast, generation of 5-HMF from
172 OPFF was recorded from 1.1 mg/g to 5.2 mg/g at pretreatment severities, log Ro of 3.1 to

3.9 (170°C, 20 min to 150°C, 240 min). Formic acid formation was correlated well with pretreatment severities and recorded highest 14.4 mg/g of OPEFB and 12.7 mg/g of OPFF at the harshest pretreatment severities (Fig. S1). Nevertheless, the degradation by-products formation in the current work are far lower in comparison to previous report (Zakaria et al., 2015a) since lower pretreatment severities are used and below than threshold limit of fermentation inhibitory level (Gong et al., 1999).

3.2 Effect of pretreatment severities on pretreated solids

Table 1 shows solid yield, pH and chemical composition of untreated and HCW-treated oil palm biomass samples at varying reaction temperature and time. In general solid yield reduction, and dropped in pH values were in line with pretreatment severities due to dissolution of hemicellulose components. Therefore, no substantial reduction of solid yield at isothermal conditions (150°C, 60-240 min) observed from both OPEFB and OPFF samples. A greater hemicellulose dissolution was more pronounced at higher treatment temperatures between 170°C to 190°C. The pH of the pretreated samples were decreased to acidic pH due to acetic acid accumulation over pretreatment severities (Fig. S1). Acid hydrolysis of pretreated solids showed gradual decreases of xylose and reached 3.3 g/ 100 g OPFF at pretreatment severity log, Ro = 3.6. Undetectable of xylose from

OPEFB samples at the harshest pretreatment severity log, $Ro = 4.0$ indicates efficient removal of hemicellulose at higher pretreatment temperature (Table 1). These results are in agreement with studies found by Bobleter (1994) who reported that hemicellulose degradation took place at or greater than 180°C . The dissolution of hemicellulose by HCW pretreatment also has resulted in an increase of glucose from cellulose which reached the highest (39.0 g/ 100 g biomass and 33.9/ 100 g biomass) at pretreatment severity log, $Ro = 3.4$ and 3.6 from OPEFB and OPFF, respectively (Table 1). An increased of Klason lignin also was recorded from both samples and correlate well with pretreatment severities. In addition, the treatment severities employed in the present study is considered suitable for both biomass since only small reduction of cellulose was observed at higher treatment severities.

3.3 Enzymatic hydrolysis

3.3.1 Individual versus combined pretreatments

The pretreated solids recovered from HCW pretreatment was subjected to enzymatic hydrolysis for 72h at 50°C . The yields of xylose and glucose corresponds to pretreatment severities are shown in Table 2. The results postulates that the yield of hydrolyzed sugars are in line with the pretreatment severities and the conversion of

209 xylose and glucose can be improved further by manipulating the pretreatment
210 temperatures and reaction times. An increased yield of glucose was more pronounced in
211 non-isothermal compared to isothermal conditions from both OPEFB and OPFF samples
212 especially at higher pretreatment temperatures due to progressive removal of
213 hemicellulose and migration of lignin from biomass cell walls (Table 1). The highest
214 glucose yield was obtained at 190°C with 72.9% and 63.5% from OPEFB and OPFF,
215 respectively. Lower glucose yield was obtained at isothermal pretreatment condition
216 probably due to partial degradation of hemicellulose and the dissolved hemicellulose
217 (xylan) might limit the adsorption of cellulase on cellulose (Qing and Wyman, 2011;
218 Zhang et al., 2012). This is supported by the results obtained which showed that the yield
219 of xylose from enzymatic hydrolysis was higher at isothermal than non-isothermal
220 condition (Table 2). Pretreatment severity was crucial in hemicellulose dissolution and
221 provides higher specific surface area that amenable for cellulase penetration (Hsu et al.,
222 2010). Therefore, the glucose yield from HCW treatment alone was below than 80% of
223 hemicellulose and cellulose conversion.

224 Previous work showed that subsequent WDM on HCW-treated OPMF sample
225 with less than 10 cycles improved substantially xylose and glucose yields (Zakaria et al.,
226 2015b). Similar approach is adopted in the present work to obtain higher yield of sugars at

227 isothermal and non-isothermal conditions from OPEFB and OPFF samples and the
228 efficiency of each treatment was evaluated and compared. Fig. 1 illustrates both xylose
229 and glucose yields are in increasing trends towards higher number of milling cycles.
230 Xylose yields are higher when OPEFB was pretreated at moderate temperature whereby
231 85% of xylose yield was obtained from HCW-pretreated samples at 150°C, 180 min (Fig.
232 1a). Xylose conversion was reduced when higher reaction temperature was imposed to
233 the OPEFB which resulted in higher degree of hemicellulose depolymerization (55.8%)
234 (Table 1). The highest cellulose conversion into glucose (90.3%) was observed when
235 pretreated by HCW at 190°C, 20 min and subsequent WDM for nine milling cycles,
236 which is two fold more than WDM pretreatment only (Fig. 1b). Slightly lower conversion
237 (87.7%) was observed when the OPEFB was pretreated at moderate temperatures (150°C
238 for 240 min). The highest xylose yield obtained from OPFF-treated solid is 66.2% at
239 190°C, 10 min (Fig. 1c). The fact that the xylose yields obtained from OPFF was lower
240 compared to OPEFB samples might attributed to efficient hemicellulose degradation to
241 XOS collected in pretreated liquids (Table S1). Complete conversion (100%) of cellulose
242 into glucose was obtained from OPFF samples at 150°C, 240 min when disk milled only
243 for five milling cycles (Fig.1d). Other pretreatment conditions recorded glucose yields
244 with less than 80%.

245 Overall, combined treatment using HCW with WDM is better than HCW
246 treatment alone in term of xylose and glucose yield. HCW pretreatment resulted in the
247 disruption of fiber's surface by `peeling off` outer layer and destruction of
248 cellulose-hemicellulose-lignin network which can be clearly seen from [Fig. S2a](#) and [Fig.](#)
249 [S2e](#) for OPEFB and OPFF [respectively, after treatment at 150°C](#) for 240 min. Partial
250 dissolution of hemicellulose at this stage should facilitate further mechanical fibrillation
251 by WDM as shown in [Fig. S2b-d](#) for OPEFB and [Fig. S2f-h](#) for OPFF. Nanoscale fibrous
252 structures up to hundreds of nanometers were observed in WDM treated samples both
253 with and without HCW treatment. [Nanoscopic](#) sizes of the fibrillated fibers obtained will
254 facilitate greater enzyme accessibility and cellulose digestibility.

255 At milder pretreatment temperature, longer retention time was required for oil
256 palm biomass samples to achieve higher conversions of xylose and glucose. The higher
257 conversions of xylose and glucose can be obtained by lowering the distance clearance
258 between 2 milling disks and increasing the milling cycles ([Hideno et al., 2012](#); [Weiqi et](#)
259 [al., 2013](#)). Higher glucose yields [were](#) obtained from *Populus* and switchgrass when
260 steam-treated at 150°C for 60 min and WDM at 17 milling cycles ([Kumagai et al., 2015](#)).
261 [Hideno et al. \(2012\)](#), who also reported that 94% of glucose yield was obtained from rice
262 straw when pretreated by HCW at 135°C for 60 min with extension of milling cycles to

15 milling passes. In another study, [Lee et al. \(2010\)](#) conducted HCW pretreatment of *Eucalyptus* at 180°C for 30 min and subsequent WDM for 10 milling cycles increased glucose production yield to 101.7%. The data obtained from the current study is in agreement with studies reported earlier ([Lee et al., 2010](#); [Hiden et al., 2012](#); [Weiqi et al., 2013](#); [Kumagai et al., 2015](#)). Regardless of types of biomass used, the proposed treatments suggested that chemical and physical structures changes during HCW influenced the subsequent pretreatment by WDM. Hence, xylose and glucose yields from both OPEFB and OPFF-pretreated solids increased due to disruption of chemical bonds after HCW pretreatment process.

3.4. Mass balance and energy consumption

[Table 3](#) shows a summary of collective results from pretreated liquids, enzyme hydrolyzates and total sugar yields from both OPEFB and OPFF samples at different pretreatment conditions. Higher recovery of monomeric sugars obtained from OPFF samples than OPEFB pretreated liquids due to chemical compositions and physical structures of fibers that react differently at pretreatment temperature. Comparable total sugars yield was observed from OPEFB samples at optimal isothermal and non-isothermal conditions with conversion yield of 88.5% and 87.6%, respectively.

Higher total sugar yields were obtained from OPFF samples with 103.3% and 89.8% at pretreatment condition of 150°C, 240 min and 190°C, 10 min, respectively. It is obvious that higher pretreatment temperature favoured higher glucose recovery and higher xylose recovery was obtained at moderate temperature with longer reaction time.

Power consumption from WDM with and without HCW treatments were calculated and presented in Fig. 2. Energy used increased in line with milling cycles and was recorded between 8.6 MJ/ kg biomass to 23.6 MJ/kg biomass and 8.3 MJ/ kg biomass to 23.5 MJ/kg biomass from OPEFB and OPFF samples, respectively. HCW-treated OPEFB samples showed lower energy consumption than WDM only pretreatment except sample with co-pretreatment with HCW at 150°C for 20 min. WDM only pretreatment of OPFF showed lower energy consumption than OPEFB samples owing to the fact that differences in the chemical composition and physical structures of each biomass. In general, slight decreases of energy consumption was observed from both samples at moderate temperatures (with increasing pretreatment reaction time) where small degree of hemicellulose degradation took place. Sharp increase of energy consumption towards higher pretreatment temperature indicates samples possesses higher viscosity probably due to high cellulose-rich samples that causes from greater hemicellulose removal (Lee et al., 2010; Kumagai et al., 2014). Cellulose-rich with fibrillated structures products

(homogenized paste form) are observed at higher pretreatment severities from both samples. The results obtained herewith are in line with previous report mentioned that higher viscosity of the pretreated samples consisted of fibrillated samples with lower hemicellulose content (Kumagai et al., 2014). Optimal total sugar yields was obtained at 15.1 MJ/kg OPEFB and 23.5 MJ/kg OPFF at 150°C, 240 min, respectively. Even though OPEFB and OPFF were pretreated at the same condition (150°C, 240 min), different time was required for each biomass to complete milling cycles. Similar results were presented by Kumagai and co-workers, (2014) where approximately 6.16 MJ/kg and 2.45 MJ/kg was needed for Hinoki cypress and eucalyptus, respectively at WDM_{5cycles} due to different in chemical composition and fiber length. Overall, xylose and glucose yields obtained from OPEFB and OPFF are comparable to study reported earlier (Zakaria et al., 2014a). Thus we conclude that the most important factor for obtaining maximal enzymatic digestibility from oil palm biomass is the expansion of the specific surface area via fibrillation of OPEFB and OPFF fibers into nanofibers. This is the first report elucidating pretreatment using HCW at isothermal and non-isothermal condition followed by wet disk milling. In addition, the present study provides progress of pretreatments developed for oil palm biomass with consideration to obtain high recovery of xylose and glucose by chemical-free approaches.

317

318 **3. Conclusion**

319   The effect of HCW and WDM pretreatment on OPEFB and OPFF were
320   successfully compared and discussed. Isothermal condition with milder reaction
321   temperature (150°C, 240 min) was the best **condition** to recover higher pentose and
322   hexose sugars. HCW pretreatment alone resulted in disruption on physical structures of
323   fibers and an increased in degree of enzymatic digestibility due to partial hemicellulose
324   removal. Subsequent WDM treatment **caused** fibrillation of oil palm biomass fibers to
325   nanoscopic **sizes further improving its enzymatic** digestibility. It can be concluded that
326   the combination of HCW and WDM pretreatment is applicable to oil palm biomass.

327

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337 **References**

338 1. Bobleter, O., 1994. Hydrothermal degradation of polymers derived from

339 plants. Prog. Polym. Sci.19, 797-841.

340 2. Cheng, J., Leu, S-Y., Zhu, J.Y., Jeffries, T.W., 2014. Ethanol production

341 from non-detoxified whole slurry of sulfite-pretreated empty fruit bunches at

342 a low cellulase loading. Bioresour. Technol. 164, 331-337.

343 3. Esteves, B.M., Pereira, H.M., 2009. Wood modification by heat treatment: A

344 review. BioResour. 4, 370-404.

345 4. Gong, C.S., Cao, N.J., Du, J., Tsao, G.T., 1999. Ethanol production from

346 renewable resources. Adv. Biochem. Eng. Biotechnol. 65, 207-241.

347 5. Hideno, A., Inoue, H., Tsukahara, K., Fujimoto, S., Minowa, T., Inoue, S.,

348 Endo, T., Sawayama, S., 2009. Wet disk milling pretreatment without

349 sulfuric acid for enzymatic hydrolysis of rice straw. Bioresour. Technol. 100,

350 2706-2711.

- 351 6. Hiden, A., Inoue, H., Yanagida, T., Tsukahara, K., Endo, T., Sawayama, S.,
352 2012. Combination of hot compressed water treatment and wet disk milling
353 for high sugar recovery yield in enzymatic hydrolysis of rice straw.
354 Bioresour. Technol. 104, 743-748.
- 355 7. Hsu, T.C., Guo, G.L., Chen, W.H., Hwang, W.S., 2010. Effect of dilute acid
356 pretreatment of rice straw on structural properties and enzymatic hydrolysis.
357 Bioresour. Technol. 101, 4907-4913.
- 358 8. Kumagai, A., Kawamura, S., Lee, S-H., Endo, T., Rodriguez, J.M., Mielenz,
359 J.R., 2014. Simultaneous saccharification and fermentation and a consolidated
360 bioprocessing for *Hinoki* cypress and *Eucalyptus* after fibrillation by steam
361 and subsequent wet-disk milling. Bioresour. Technol. 162, 89-95.
- 362 9. Kumagai, A., Wu, L., Iwamoto, S., Lee, S-H., Endo, T., Rodriguez, J.M.,
363 Mielenz, J.R., 2015. Improvement of enzymatic saccharification of *Populus*
364 and switchgrass by combined pretreatment with steam and wet disk milling.
365 Renew. Energy. 76, 782-789.
- 366 10. Lee, S.H., Chang, F., Inoue, S., Endo, T., 2010. Increase in enzyme
367 accessibility by generation of nanospace in cell wall supramolecular
368 structure. Bioresour. Technol. 101, 7218-7223.

- 369 11. Lee, K.T., Ofori-Boateng, C., 2013. Oil palm biomass as feedstock for
370 biofuel production. Sustainability of biofuel production from oil palm
371 biomass, green energy and technology. 77-106. Springer.
- 372 12. Mosier, N.S., Wyman, C., 2005. Features of promising technologies for
373 pretreatment of lignocellulosic biomass. Bioresour. Technol. 96, 673-686.
- 374 13. Ofori-Boateng, C., Lee, K. T., 2014. Sono-assisted organosolv/H₂O₂
375 pretreatment of oil palm (*Elaeis guineensis* Jacq.) fronds for recovery of
376 fermentable sugars: Optimization and severity evaluation. Fuel. 115, 170-178.
- 377 14. Overend, R.P., Chornet, E., 1989. Steam and aqueous pretreatments: are they
378 a prehydrolyses? In: Wood Process. Util. Elsevier, New York, pp. 395-400.
- 379 15. Palamae, S., Palachum, W., Chisti, Y., Choorit, W., 2014. Retention of
380 hemicellulose during delignification of oil palm empty fruit bunch (EFB)
381 fiber with peracetic acid and alkaline peroxide. Biomass. Bioenerg.
382 66,240-248.
- 383 16. Qing, Q., Wyman C.E., 2011. Supplementation with xylanase and
384 b-xylosidase to reduce xylo-oligomer and xylan inhibition of enzymatic
385 hydrolysis of cellulose and pretreated corn stover. Biotechnol. Biofuel. 4, 18.

- 386 17. Sabiha-Hanim, S., Mohd Noor, M.A., Rosma, A., 2011. Effect of
387 autohydrolysis and enzymatic treatment on oil palm (*Elaeis guineensis*
388 *Jacq.*) frond fibres for xylose and xylooligosaccharides production.
389 Bioresour. Technol. 102, 1234-1239.
- 390 18. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008.
391 Determination of Sugars, Byproducts, and Degradation Products in Liquid
392 Fraction Process Samples. NREL/TP-510-42623, Laboratory Analytical
393 Procedure (LAPs). National Renewable Energy Laboratory, Golden, CO.
- 394 19. Weiqi, W., Shubin, W., Ligu, L., 2013. Combination of liquid hot water
395 pretreatment and wet disk milling to improve the efficiency of the enzymatic
396 hydrolysis of eucalyptus. Bioresour. Technol. 128, 725-730.
- 397 20. Zakaria, M.R., Fujimoto, S., Hirata, S., Hassan, M.A., 2014a. Ball milling
398 pretreatment of oil palm biomass for enhancing enzymatic hydrolysis. Appl.
399 Biochem. Biotechnol. 173, 1778-1789.
- 400 21. Zakaria, M.R., Hirata, S., Hassan, M.A., 2014b. Combined pretreatment
401 using alkaline hydrothermal and ball milling to enhance enzymatic
402 hydrolysis of oil palm mesocarp fiber. Bioresour. Technol. 169, 236-243.

22. Zakaria, M.R., Hirata, S., Hassan, M.A., 2015a. Hydrothermal pretreatment enhanced enzymatic hydrolysis and glucose production from oil palm biomass. *Bioresour. Technol.* 176, 142-148.

23. Zakaria, M.R., Norraahim, M.N.F., Hirata, S., Hassan M.A., 2015b. Hydrothermal and wet disk milling pretreatment for high conversion of biosugars from oil palm mesocarp fiber. *Bioresour. Technol.* 181, 263-269.

24. Zhang, J., Tang, M., Viikari, L., 2012. Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases. *Bioresour. Technol.* 121, 8-12.

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Table 1 Solid yield and chemical composition of untreated and HCW- treated oil palm biomass samples at varying reaction temperature and time.

*Values presented are from triplicates analyses.

Table 2 Effect of hot compressed water on yields of xylose and glucose at various pretreatment conditions.

Table 3 Mass balance of OPEFB and OPFF obtained from pretreated liquid and enzyme

hydrolyzates of pretreatment conditions.

^aXylose (combination of monomeric and XOS).

^bPretreated solids after 9 passes of WDM, 5% substrate loading, 10 FPU/ g-substrate,

50°C, 72 h.

FIGURE CAPTIONS

Fig. 1 Yields of xylose and glucose from OPEFB (a, b) and OPFF (c, d), respectively at

various pretreatment severities and milling cycles.

Fig. 2 Energy consumption calculated after 9 passes of WDM from OPEFB and OPFF.

Table 1 Solid yield and chemical composition of untreated and HCW- treated oil palm biomass samples at varying reaction temperature and time.

	Pretreatment severity, log (Ro)	Solid yield (%)	pH	Compositions (g/100 g biomass)		
				Glucose	Xylose	Klason Lignin
<i>Untreated OPEFB</i>	-	100	6.5	31.7 ± 4.7	23.2 ± 2.7	19.2 ± 0.5
HCW 150°C, 60 min	3.3	85.4	5.5	33.0 ± 7.2	15.9 ± 3.8	20.7 ± 2.4
HCW 150°C, 120 min	3.6	84.8	5.5	33.9 ± 9.7	15.9 ± 5.4	20.8 ± 0.7
HCW 150°C, 180 min	3.7	81.7	5.4	33.9 ± 9.4	15.0 ± 4.3	24.5 ± 1.2
HCW 150°C, 240 min	3.9	81.9	5.2	34.8 ± 0.9	13.9 ± 1.2	23.9 ± 0.7
HCW 150°C, 20 min	2.8	87.8	6.0	35.1 ± 2.7	18.2 ± 1.2	23.1 ± 1.1
HCW 170°C, 20 min	3.4	84.7	4.4	39.0 ± 2.4	14.2 ± 3.1	22.9 ± 1.6
HCW 190°C, 20 min	4.0	72.2	4.1	37.2 ± 0.9	0.0 ± 0.0	25.3 ± 1.1
<i>Untreated OPFF</i>	-	100	6.5	34.5 ± 2.7	21.5 ± 1.8	14.7 ± 0.2
HCW 150°C, 60 min	3.3	79.3	5.0	28.6 ± 4.2	14.4 ± 4.6	16.2 ± 0.7
HCW 150°C, 120 min	3.6	79.1	4.8	27.8 ± 4.2	11.0 ± 2.1	17.2 ± 0.4
HCW 150°C, 180 min	3.7	77.6	4.6	32.4 ± 9.4	9.6 ± 3.4	18.3 ± 1.1
HCW 150°C, 240 min	3.9	76.2	4.4	33.7 ± 2.4	5.9 ± 2.4	23.1 ± 0.6
HCW 150°C, 10 min	2.5	67.7	5.6	30.0 ± 1.2	16.8 ± 0.3	17.4 ± 0.4
HCW 170°C, 10 min	3.1	63.8	4.1	33.2 ± 1.3	12.9 ± 0.9	20.1 ± 0.8
HCW 190°C, 10 min	3.6	63.5	3.8	33.9 ± 1.1	3.3 ± 0.3	27.2 ± 0.3

*Values presented are from triplicates analyses.

Table 2 Effect of hot compressed water on yields of xylose and glucose at various pretreatment conditions.

Pretreatments	Pretreatment severity, log (<i>Ro</i>)	Yield of hydrolyzed sugars	
		Xylose (%)	Glucose (%)
<i>OPEFB</i>	-	8.2 ± 1.1	1.5 ± 0.9
HCW 150°C, 60 min	3.3	27.5 ± 0.5	32.0 ± 0.9
HCW 150°C, 120 min	3.6	35.1 ± 1.6	36.0 ± 1.3
HCW 150°C, 180 min	3.7	41.4 ± 1.3	36.2 ± 1.2
HCW 150°C, 240 min	3.9	47.5 ± 0.6	40.0 ± 0.5
HCW 150°C, 20 min	2.8	23.1 ± 0.6	29.4 ± 1.2
HCW 170°C, 20 min	3.4	38.8 ± 1.0	41.6 ± 0.3
HCW 190°C, 20 min	4.0	40.8 ± 0.9	72.9 ± 2.2
<i>OPFF</i>	-	11.5 ± 1.2	36.6 ± 1.5
HCW 150°C, 60 min	3.3	16.4 ± 1.8	32.9 ± 4.4
HCW 150°C, 120 min	3.6	22.4 ± 0.2	45.8 ± 1.6
HCW 150°C, 180 min	3.7	27.2 ± 2.5	48.2 ± 4.0
HCW 150°C, 240 min	3.9	31.6 ± 1.2	61.8 ± 1.7
HCW 150°C, 10 min	2.5	17.2 ± 1.1	33.1 ± 2.6
HCW 170°C, 10 min	3.1	16.8 ± 0.8	37.0 ± 2.7
HCW 190°C, 10 min	3.6	29.7 ± 0.6	66.5 ± 0.0

Table 3 Mass balance of OPEFB and OPFF obtained from pretreated liquid and enzyme hydrolyzates of pretreatment conditions.

Pretreatments	Pretreated liquid ^a (g/ 100 g of biomass)		Enzyme hydrolyzate ^b (g/ 100 g of biomass)		Pretreated liquid + enzyme hydrolysate (g/ 100 g of biomass)		Total sugars	Total sugars
	Xylose ^a	Glucose	Xylose	Glucose	Xylose	Glucose		(%)
<i>OPEFB</i>								
HCW 150°C, 60 min	0.4 ± 0.0	0.3 ± 1.2	9.3 ± 1.0	17.1 ± 6.6	9.7 ± 1.0	17.4 ± 7.8	27.1 ± 8.8	57.3
HCW 150°C, 120 min	0.3 ± 0.0	0.5 ± 0.0	11.4 ± 0.9	19.6 ± 3.7	11.7 ± 0.9	20.1 ± 3.7	31.8 ± 4.7	67.2
HCW 150°C, 180 min	0.3 ± 0.0	1.6 ± 0.4	13.3 ± 3.7	25.0 ± 3.1	13.6 ± 3.7	26.6 ± 3.5	40.2 ± 7.2	85.0
HCW 150°C, 240 min	0.3 ± 0.0	3.0 ± 0.2	16.1 ± 4.0	35.0 ± 1.4	16.4 ± 4.0	38.0 ± 1.6	54.4 ± 5.6	88.5
HCW 150°C, 20 min	0.2 ± 0.0	0.3 ± 0.0	13.2 ± 1.7	22.8 ± 5.1	13.4 ± 1.7	23.1 ± 5.1	36.5 ± 6.8	59.3
HCW 170°C, 20 min	0.2 ± 0.0	1.2 ± 0.0	15.1 ± 0.5	26.8 ± 0.7	15.3 ± 0.5	28.0 ± 0.7	43.3 ± 1.2	70.4
HCW 190°C, 20 min	0.3 ± 0.0	5.5 ± 0.4	12.1 ± 1.2	36.0 ± 2.0	12.4 ± 1.2	41.5 ± 2.4	53.9 ± 3.6	87.6
<i>OPFF</i>								
HCW 150°C, 60 min	5.8 ± 0.2	2.0 ± 0.2	6.8 ± 0.7	26.3 ± 0.6	12.6 ± 0.9	28.3 ± 0.8	40.9 ± 1.7	69.4
HCW 150°C, 120 min	3.1 ± 0.7	1.9 ± 0.3	8.0 ± 0.6	23.7 ± 0.2	11.1 ± 1.3	25.6 ± 0.5	36.7 ± 1.8	62.3
HCW 150°C, 180 min	4.4 ± 0.3	3.2 ± 0.7	8.2 ± 0.1	25.3 ± 0.2	12.6 ± 0.4	28.5 ± 0.9	41.1 ± 1.3	70.0
HCW 150°C, 240 min	4.2 ± 0.0	4.9 ± 0.0	8.6 ± 0.5	43.4 ± 0.1	12.8 ± 0.5	48.3 ± 0.1	61.1 ± 0.6	103.7
HCW 150°C, 10 min	3.8 ± 0.2	0.8 ± 0.0	6.9 ± 0.1	25.4 ± 1.5	10.7 ± 0.3	26.2 ± 1.5	36.9 ± 1.8	62.3
HCW 170°C, 10 min	3.7 ± 0.9	1.8 ± 0.6	11.0 ± 0.2	30.5 ± 0.2	14.7 ± 1.1	32.3 ± 0.8	47.0 ± 1.9	79.8
HCW 190°C, 10 min	2.9 ± 0.1	5.0 ± 0.1	11.2 ± 0.5	33.8 ± 0.6	14.1 ± 0.6	38.8 ± 0.7	52.9 ± 1.3	89.8

^aXylose (combination of monomeric and XOS).

^bPretreated solids after nine (9) passes of WDM, 5% substrate loading, 10 FPU/ g-substrate, 50°C, 72 h.

Fig. 1 Yields of xylose and glucose from OPEFB (a, b) and OPFF (c, d), respectively at various pretreatment severities and milling cycles.

Fig. 2 Energy consumption calculated after nine (9) passes of WDM from OPEFB and OPFF.