Hydrothermal and wet disk milling pretreatment for high conversion of biosugars from oil palm mesocarp fiber

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Abstract

Eco-friendly pretreatment methods for lignocellulosic biomass are being developed as alternatives to chemical based methods. Superheated steam (SHS), hot compressed water (HCW) and wet disk milling (WDM) were used individually and with combination to partially remove hemicellulose and alter the lignin composition of recalcitrant structure of oil palm mesocarp fiber (OPMF). The efficiency of the pretreatment methods were evaluated based on the chemical compositions altered, SEM analysis, power consumption and degree of enzymatic digestibility. Hemicellulose removal (94.8%) was more pronounced under HCW compared to SHS, due to maximal contact of water and production of acetic acid which enhanced further degradation of hemicellulose. Subsequent treatment with WDM resulted in defibrillation of OPMF and expansion of the specific surface area thus increasing the conversion of cellulose to glucose. The highest glucose yield was 98.1% (g/g-substrate) when pretreated with HCW (200°C, 20 min) and WDM which only consumed 9.6 MJ/kg of OPMF.

Keywords: Oil palm mesocarp fiber, hydrothermal pretreatment, hot compressed water, wet disk milling pretreatment of biomass, enzymatic hydrolysis.

1. Introduction

Malaysia annually generates 6.93 million tonnes of oil palm biomass (dry basis) ([Nazir et al., 2013](#)) which provides potential bioresources for the conversion into value-added products such as chemical feedstocks, biosugars, biofuels, bioplastic, cellulose and composite production

(Zahari et al., 2014). The other major resource being wasted is excess steam produced through burning of oil palm oil biomass (Yoshizaki et al., 2013). The integration and effective utilization of biomass and excess steam are believed to provide eco-processes and green products towards a sustainable oil palm industry. Oil palm biomass has attracted attention of researchers due to its potential utilization for sugar recovery. Oil palm empty fruit bunches (OPEFB), oil palm mesocarp fiber (OPMF) and oil palm frond fiber (OPFF) can be obtained abundantly throughout the year. Extensive research has been done on conversion of these materials to ethanol by hydrolysis of cellulose to fermentable sugars (Bakar et al., 2012; Zahari et al., 2014). The presence of hemicellulose and lignin hinders the access of the cellulase to cellulose, thus resulting in low efficiency of the hydrolysis (Sun and Cheng, 2002). Previous reports have shown that, by removing hemicellulose and lignin (Zakaria et al., 2014b), reduction of cellulose crystallinity (Hiden et al., 2009; Silva et al., 2010) and increase of porosity (Lee et al., 2010) from pretreatment processes can substantially improve the enzymatic hydrolysis.

Selection of suitable pretreatment is necessary to get rid of this recalcitrance prior to saccharification for bioethanol and biofuels production (Mosier and Wyman, 2005; Weiqi et al., 2013). Efficient utilization of lignocellulose requires an initial pretreatment step to minimize the barrier caused by hemicellulose and lignin, thus higher enzymatic digestibility can be achieved (Vancov et al., 2012). Several pretreatments can be applied to oil palm biomass such as chemical, mechanical and hydrothermal treatment in order to overcome this hindrance. Pretreatment involving hot compressed water (HCW), liquid hot water (LHW), steam, and superheated steam (SHS) are gaining interest recently, as these treatments are more environmental friendly, and time saving for enzymatic saccharification of natural biomass (Yu et al., 2011; Inoue et al., 2008; Weiqi et al., 2013 Yunos et al., 2012; Nik Mahmud et al., 2013).

Superheated steam is unsaturated (dry) steam generated by the addition of sensible heat to saturated (wet) steam (Bahrin et al., 2012). Pretreatment using SHS has several advantages such as improved energy efficiency, higher drying rates, use of an air-free environment, and reduced environmental impact when condensate is reused (Nik Mahmud et al., 2013). Moreover, SHS pretreatment is a cost-effective method for large-scale purpose and it is one of the eco-friendly approaches due to the utilization of excess steam produced at the palm oil mill. However, SHS or other hydrothermal treatment alone does not produce a high yield of hydrolysed sugars (Nik Mahmud et al., 2013; Bahrin et al., 2012; Zakaria et al., 2014b). Combination of hydrothermal pretreatment with other mechanical pretreatment has been reported to substantially improve sugar yields (Inoue et al., 2008; Hiden et al., 2012).

Comminution processes such as planetary/attrition ball milling and wet disk milling have resulted in reduction of particle size, increased surface area, increased pore volume and reduced crystallinity (CrI) of cellulose, thus enhancing the enzymatic digestibility of biomass (Hiden et al., 2009; Silva et al., 2010; Zakaria et al., 2014a). There have been very few reports of pretreatment technologies tested on OPMF. The effect of combined pretreatment using alkaline hydrothermal and ball milling to enhance enzymatic hydrolysis of OPMF was reported (Zakaria et al., 2014b). Even though high recovery of xylose and glucose were obtained, higher energy consumption was recorded from ball mill process. Recently, wet disk milling (WDM) has been used to produce relatively low levels of inhibitors and increased degree of defibrillation which created more space between microfibrils, thus enhanced the enzymatic hydrolysis of the fiber (Lee et al., 2010; Zhang et al., 2013). Wet disk milling should be combined with other treatments in order to facilitate the enzyme accessibility of the fiber (Gao et al., 2012). Combination of hydrothermal (LHW and or HCW) with WDM pretreatment on rice straw (Hiden et al., 2012)

and eucalyptus (Weiqi et al., 2013) have shown an improvement of sugar yield compared to hydrothermal pretreatment alone. Compared to previous studies on combination of HCW and WDM the present study highlighted a suitability of OPMF as raw material for high glucose conversion comparable to rice straw and eucalyptus chips. In addition, a bigger space gap between upper and lower grinders (150-300 μm) was adopted in the present study compared to 20-40 μm (Hiden et al., 2012; Weiqi et al., 2013) in order to avoid overheating of samples and additional maintenance cost of grinders.

Therefore, in this work we investigate the suitability of individual hydrothermal (SHS and HCW) and WDM pretreatment alone and its combination on OPMF in order to understand the mechanism involved for each method used that affects biomass structure and cellulose properties. The effect of SHS, HCW and WDM on size reduction, specific surface area, morphological analysis and energy consumption are compared and discussed.

2. Methods

2.1. Raw materials

The OPMF used in this study was obtained from Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia. The samples were sun dried and cut by milling cutters to size <2 mm. The ground samples were stored in *vacuo* at room temperature (24°C) prior to further analysis.

2.2. Hydrothermal pretreatment

2.2.1. Superheated steam (SHS)

The SHS pretreatment of OPMF was carried out using a lab scale superheated steam machine (Naomoto Co., Tokyo, Japan) with outer and inner oven sizes of (W70 cm x D60 cm x

H60 cm) and (W20 cm x D30 cm x H20 cm), respectively. The SHS oven consists of a stainless steel heating chamber and a heater kind of boiler. Steam was generated by electric steam generator (NBC-2070R model, Naomoto Co., Tokyo Japan) with steam evaporation and steam pressure at 10.5 kg/h and 0.3 MPa, respectively. Tap water was pretreated by auto-softener machine (Mk-6J model, Maruyama Co., Ltd., Tokyo Japan) and controlled by valve timer. Approximately 0.9-1.0 kg of ion exchange resin- salt was used per regeneration of soft-water. The selection of pretreatment conditions by SHS for OPMF was based on the best treatment condition reported in the literature ([Nordin et al., 2013](#)), conducted at temperature ranging from 190°C to 230°C for 60 min.

2.2.2. *Hot compressed water (HCW)*

Hot compressed water pretreatment was performed using a 1 L stainless steel autoclave (Nitto Koatsu Co., Tsukuba, Japan) as reported earlier ([Hideno et al., 2009](#)) with slight modification. Oil palm mesocarp fiber (50 g) and water (500 mL) were added to autoclave system and mixed at 250-300 rpm and the samples mixture were heated to a reaction temperature range from 160°C to 200°C at a heating rate of 2-3°C/ min and maintained for 20 min. The autoclave was left and cooled down at room temperature prior to further process. The pretreated solids were separated from slurries by using Advantec No. 2 membrane filter and rinsed with distilled water until neutral pH.

2.2.3. *Wet disk milling (WDM)*

Wet disk milling was performed using Supermass colloidier MKZA10 (Masuko Saangyo Co., Ltd., Saitama, Japan) as reported earlier ([Hideno et al., 2009](#)) with slight modification.

Briefly, the ceramic nonporous disk grinders was adjusted for a clearance of 150- 300µm between the upper and lower grinders and revolved at 1800 rpm. Fifty (50) g of untreated (< 2 mm) and hydrothermally pretreated OPMF (SHS and HCW) solids were soaked in water (1 L) for 2-3 days prior to WDM and the operation was repeated for 2-20 times. The pretreated slurry was **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

Unless otherwise stated, 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 expressed by the obtained sugars (mg sugars/g materials) or sugar yield as calculated using Eq. (1):

$$\text{Sugar yield (\%)} = \left[\frac{\text{weight of monomeric sugars after enzymatic hydrolysis}}{\text{total monomeric sugars after hydrolysis using H}_2\text{SO}_4} \right] \times 100 \quad (1)$$

2.4. Energy consumption in pretreatments

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

2.5. Analytical methods

Compositional analysis of untreated and pretreated OPMF was determined by a method recommended by Teramoto et al. (2008). Detection of sugars was performed using high-performance liquid chromatography (HPLC) equipped with a refractive index detector (RID-10A, Shimadzu, Japan) using an Aminex HPX-87P column (7.8x30 cm I.D., BioRad, USA) with a Carbo-P micro-guard cartridge. The column oven was set at 80°C, and samples were eluted at 0.60 mL/min with water (Inoue et al., 2008).

Morphological analysis was conducted by using field emission scanning electron microscope (FE-SEM; S-3400 and S-4800, Hitachi, Japan) at 1.0 -15 kV. The untreated and pretreated OPMF was sputtered with Pt-Pd for 100 s (ion sputter, Hitachi, Japan). Combined hydrothermal and WDM pretreated- samples were serially diluted to appropriate concentrations and the solids were separated by 47 mm PTFE membrane filter with pore size, 1.0 µm (Advantec, Japan). The solids were rinsed with ethanol and soaked in butyl alcohol for 1 min. Samples were vacuum dried at room temperature prior to SEM analysis. This step was performed to observe the defibrillation of OPMF samples after WDM.

The untreated and pretreated samples were subjected to specific surface area measurement and was performed according to previous report (Zakaria et al., 2015).

3. Results and discussion

3.5. Chemical compositions of hydrothermally- treated OPMF

Superheated steam pretreatment, hot compressed water pretreatment, wet disk milling pretreatment were performed individually and in their combination to reduce the natural recalcitrant of lignocellulosic oil palm biomass and the summary of experimental design is

shown in [Fig. 1](#). The chemical compositions of untreated and hydrothermally pretreated OPMF as function of pretreatment severities are shown in [Table 1](#). OPMF is mainly comprised of cellulose (25.4%), followed by lignin (24.6%) and hemicellulose (21.2%). The chemical compositions of raw OPMF determined in this study were comparable to that reported by [Zakaria et al. \(2014b\)](#) except for hemicellulose content which recorded lower in this study. Hemicellulose obviously was affected by hydrothermal pretreatment and its content decreased in line with increased treatment severity (temperature and time). About 12.3% and 1.1% of hemicellulose content were recorded after being treated with SHS (210°C, 60 min) and HCW (200°C, 20 min), respectively. In other words, approximately 42.0% and 94.8% of hemicellulose had been degraded by SHS and HCW, respectively. This finding was in agreement with study reported by [Nordin et al. \(2013\)](#) showed reduction of hemicellulose content was about 49.2% after SHS-pretreated at 210°C for 60 min. Even though SHS pretreatment showed an ability to remove hemicellulose from the fiber at the same time, degradation of cellulose was recorded when the temperature was higher than 200°C and similar trend of cellulose degradation was observed from a study by [Nordin et al. \(2013\)](#). In addition, [Nik Mahmud and co-workers \(2013\)](#), reported that only 15.6% of weight lost was obtained after OPMF was SHS-pretreated at the same condition. These results clearly indicated that HCW was an efficient pretreatment in hemicellulose removal due to maximal contact between liquid and solid biomass. Furthermore, acetic acid produced during de-acetylation of hemicellulose dissolution improved the hydrolysis process ([Mosier and Wyman, 2005](#); [Lee et al., 2010](#); [Weiqi et al., 2013](#); [Xiao et al., 2014](#)). The cellulose content recorded higher after SHS pretreatment at 190°C, 60 min (34.7%) and reduced gradually towards higher treatment temperatures. Meanwhile, cellulose content increased steadily from 31.7%-38.1% after being treated by HCW for 20 min at temperature range from

160-200°C. The HCW pretreatment gives higher recovery of cellulose than SHS pretreatment, due to effective removal of hemicellulose and lignin from the material which led to increase efficiency of enzyme penetration to cellulose. [Zakaria and co-workers \(2014b\)](#) reported that hydrothermally-pretreated OPMF samples had removed about 65% of hemicellulose and gave substantial increased in glucose conversion. Higher hemicellulose dissolution obtained in this study might be due to smaller size of OPMF samples used (<2mm) compared to original size from previous report ([Zakaria et al., 2014b](#)). Hemicellulose and lignin have been reported to hinder the efficiency of enzymatic saccharification by limiting the cellulase accessibility to cellulose and by adsorbing enzymes ([Mosier and Wyman, 2005](#); [Miura et al., 2012](#)). Several researchers have reported that lignin removal enhanced hydrolysability of lignocellulosic materials ([Miura et al., 2012](#); [Barros et al., 2013](#)). The increment of lignin composition from both SHS and HCW pretreatments were due to the destruction of hydroxyl groups in hemicellulose ([Nordin et al., 2013](#)) and the formation of pseudolignin from carbohydrate degradation ([Pu et al., 2013](#); [Zakaria et al., 2014b](#)). The effect of individual and combined pretreatments on morphology and structural changes of OPMF will be discussed in a later section.

3.2. SEM analysis of structural changes of OPMF

Individual hydrothermal (SHS and HCW) and WDM pretreatment and their combinations have resulted in morphological changes of OPMF as evaluated by SEM. [Fig. S1a](#) shows that the untreated OPMF has a rigid and intact surface which impedes the penetration of degradation enzymes to the targeted compounds. A small morphological alteration was observed after being treated by SHS at 200°C, 60 min ([Fig. S1b](#)). In contrast, [Fig. S1c](#) shows a higher degree of surface morphology alteration when OPMF was pretreated with HCW, whereas efficient

dissolution of hemicellulose may result in peeling-off of outer layer of fiber (Zakaria et al., 2014b). Wet disk milling was performed to reduce particle size and increased specific surface area. Fig. S1d shows the SEM image of WDM treated-OPMF samples after 20 operation cycles. As shown in Fig. S1e-f, there is no obvious difference from combined hydrothermal (SHS and HCW) and WDM in comparison to WDM pretreatment alone. Serial dilution was performed, washed several times with ethanol as described in the methodology section in order to observe the differences between SHS and HCW-WDM pretreatment. The degree of defibrillation and particle size reduction played a very important part to enhance the conversion of hydrolysed sugars from lignocellulosic material. From the morphological analysis, defibrillation of OPMF occurred rapidly as the amount of operation cycle of WDM increased. Well defibrillated fibers with a diameter size smaller than 50 nm was observed for OPMF treated with HCW as shown in Fig. S1h-i. These results indicate the defibrillation of OPMF by HCW pretreatment is more pronounced than defibrillation by SHS pretreatment (Fig. S1g) probably due to greater hemicellulose removal which resulted in increased specific surface area of the particles. Partial removal of hemicellulose and alteration or migration of lignin from the cellulose-hemicellulose-lignin matrix may have caused OPMF to be more ductile and led to loosen packing of cellulose microfibrils (Lee et al., 2010). Thus, it will ease the WDM pretreatment by reducing the particle size and increased the efficiency of hydrolytic enzyme penetration to the fiber (Weiqi et al., 2013).

3.3. Enzymatic hydrolysis of untreated and pretreated OPMF

Successful saccharification process is heavily dependent on the efficiency of pretreatment applied to improve cellulose accessibility, which is the principal factor to increase the conversion

degree of cellulose to glucose ([Vancov et al., 2012](#)). The efficiency of the hydrothermal pretreatment (SHS and HCW) and WDM were assessed by the degree of enzymatic digestibility. Yields of hydrolysed sugars obtained from untreated, SHS and HCW- pretreated OPMF are shown in [Table 2](#). Xylose and glucose conversion yield from untreated OPMF samples (< 2mm size) were 5.8% and 16.6%, respectively. Xylose conversion yields obtained from SHS and HCW were 6.6-10.7%, respectively while glucose conversion yield from SHS pretreatment alone achieved the highest at 16.4% after being treated for 60 min at 190°C. However, glucose conversion yield declined after pretreatment at 190°C, probably due to 'over cooking' of biomass, as supported in [Table 1](#), showing reduction in cellulose content as the pretreatment temperature increased over 200°C. In contrast, higher glucose conversion was recorded when OPMF was pretreated by HCW (21.4-62.9%) from 160-200°C for 20 min. [Nik Mahmud and co-workers \(2013\)](#) found that SHS-pretreated OPMF at 180°C for 60 min has improved enzymatic hydrolysis by 58.3%. Due to lack of information in the literature regarding SHS pretreatment of biomass particularly OPMF, we may conclude that SHS pretreatment appeared to be unsuitable for OPMF which resulted in low yields of hydrolysed sugars as compared to the untreated fiber. Even though SHS pretreatment caused a partial degradation of hemicellulose, nevertheless SHS pretreatment only caused a small effect on the chains and minimal damage to the cellulose-hemicellulose-lignin network. Superheated steam is known as an unsaturated steam produced which has a limitation on the water contact with fibers. In contrast, HCW pretreatment is more effective pretreatment due to the ability of water to get maximal contact with fibers compared to SHS pretreatment that resulted in greater hemicellulose dissolution as supported in [Table 1](#).

[Fig. 2](#) illustrates xylose and glucose conversion yield from WDM pretreatment only (labelled as raw) and combined hydrothermal (SHS and / or HCW) with WDM pretreatment at

different pretreatment severities and samples were taken at certain milling cycles for the determination sugars. In general, xylose and glucose yield were increased in line with milling cycles. In SHS-treated OPMF, the highest xylose and glucose yield were obtained at 57.0% and 64.7%, respectively when pretreated at 200°C, for 60 min with subsequent WDM_{cycles13} and plateaued afterwards, probably due to the degradation of sugars by heat which had been produced from the fractionation of milling stones when the operation milling cycles is increased. (Fig. 2a-b). These results showed that combined pretreatment using SHS and subsequent WDM substantially improved the conversion yield of xylose and glucose compared to SHS and WDM pretreatment alone. Fig. 2c-d shows HCW-WDM pretreatment of OPMF substantially increased the efficiency of enzymatic saccharification than SHS-WDM treated OPMF. This can be explained by higher specific surface area (SSA) obtained from HCW-treated OPMF samples. It was observed that the SSA of HCW and HCW-WDM treated OPMF was increased substantially. The SSA of untreated OPMF was 1.1 m²/g and the HCW-treated OPMF samples at 160°C, 180°C, and 200°C were recorded at 3.9 m²/g, 12.3 m²/g, and 21.2 m²/g, respectively. This finding is in agreement with previous study reported hydrothermal pretreatment of oil palm biomass improved SSA and enhanced enzymatic digestibility (Zakaria et al., 2015). The SSA was recorded higher at 20.7 m²/g, and 31.5 m²/g from HCW-treated samples at 180°C and 200°C, respectively when the samples were pretreated further with WDM for nine cycles (passess). The highest xylose yield recorded from pretreated solid was 55.1% when the OPMF was HCW-pretreated at 180°C with subsequent WDM for 5 milling cycles. The xylose yield was recorded the lowest at highest pretreatment temperature (200°C) due to almost complete dissolution of hemicellulose as supported in Table 1. Meanwhile, the highest glucose yield obtained was higher than 98% from HCW-WDM treated OPMF at 200°C, 20 min after WDM only for 5 operation

cycles (Fig. 2d). The glucose yield obtained at this stage was comparable to previous work reported by Zakaria et al. (2014b) which used alkaline hydrothermal and ball milling pretreatment on OPMF. From this finding, we may conclude that WDM and SHS-WDM pretreatment did not facilitate the defibrillation of the OPMF fibers, as only small removal of hemicellulose and morphological changes were observed (Fig. S1). In this case WDM favoured reduction of the fiber's particle size than defibrillation (Fig. S2). Hemicellulose removal by HCW pretreatment has been reported to generate nanoscopic pores between cellulose microfibrils bundles which can loosen the network structure of polymer matrix (Lee et al., 2010; Chang et al., 2012). In another report, delignification of lignocellulosic biomass and subsequent WDM resulted in higher degree of defibrillation, reduction of milling cycles and increase specific surface area (Barros et al., 2013; Miura et al., 2012; Gao et al., 2012). The removal of hemicellulose and migration/removal of lignin from the cellulose-hemicellulose-lignin polymer matrix eased the milling treatment in order to reduce the biomass particle size and defibrillation into nanofibers. Thus, it could enhance the efficiency of hydrolytic enzyme penetration to the fiber (Lee et al., 2010; Weiqi et al., 2013). It is worth to mention that, enzymatic digestibility was not affected by the accumulation of Klason lignin from the hydrothermal processes. The present study was in agreement with data reported by Lee et al. (2010). Hence, defibrillation of OPMF fiber was a key factor in maximal cellulose attack on cellulose.

3.4. Energy consumption

Although mechanical milling process was one of the most effective methods to increase specific surface area and enzymatic accessibility, combined pretreatment of the lignocellulosic material will reduce the energy consumption (Barakat et al., 2014). In other report, economic

analysis indicated that enzyme cost for ethanol production is reduced by 19-67% by WDM with HCW treatment (Hideno et al., 2012). Table 3 shows the comparative analysis of yield of hydrolyzed sugars and energy consumption from combined WDM with SHS and HCW pretreatments at different pretreatment severities with other studies. Wet disk milling pretreatment alone required about 18.9 MJ/kg of OPMF and recorded higher in comparison to rice straw, sugarcane bagasse and sugarcane straw (Hideno et al., 2009; Silva et al., 2010). Therefore, WDM pretreatment required lower energy consumption compared to ball milling pretreatment (Zakaria et al., 2014a; Zakaria et al., 2014b). It was observed that the energy requirement was decreased when OPMF was induced with hydrothermal pretreatment prior to WDM treatment, that resulted in energy consumption of 5.1 MJ/kg and 17.5 MJ/kg of OPMF when pretreated with SHS (210°C, 60 min) and HCW (200°C, 20 min), respectively. The yield of hydrolysed sugars correlated with the WDM processing cycles. Based on the current study, the energy consumption depended on the types of pretreatment, as partial removal of hemicellulose and migration of lignin are sufficient to weaken the matrix of lignocellulosic network between cellulose microfibrils. SHS-treated OPMF required a low energy (10.3 MJ/kg biomass) as compared to HCW pretreatment (17.5 MJ/kg biomass) at WDM cycle 9. It was also observed that, the higher amount of energy consumption of WDM depended on the viscosity of the sample where HCW-treated OPMF was found to have higher degree of viscosity than SHS-treated OPMF. SHS- treated OPMF has higher degree of grindability as a smaller particle size obtained after WDM as compared to HCW-treated OPMF. 'Over cooking' of OPMF samples in SHS drying oven may have influenced this phenomenon even though the same liquid to solid (L:S) ratio was applied to both SHS and HCW samples. Considering the higher specific surface area and degree of defibrillation from HCW-WDM process, lower enzyme loading may apply to

saccharification stage (Hideno et al., 2012). We may hypothesized that the energy consumption from WDM processes may varied from types of biomass, pretreatments applied prior to WDM, L: S applied and milling cycles. It is worth to mention that the developed pretreatment method on OPMF showed a promising technology by considering low energy consumption, efficient treatment with high glucose yield and no chemical catalyst used.

3.5. *Material balance*

The results obtained from the best pretreatment methods and enzymatic hydrolysis were used to develop a process material balance as shown in Fig. 3. All data reported were based on 100 g of initial dry OPMF. About 65.0 g of pretreated solids can be recovered after HCW pretreatment (180°C, 20 min) which is mainly contained glucan and ligin. About 0.5 g glucose, 7.6 g xylose, 0.9 g galactose, 1.0 g arabinose, 0.1 g acetic acid, 0.3 g furfural, and 0.3 g formic acid can be recovered in the prehydrolyzate from HCW pretreatment, respectively. More than 50% of total xylose (monomeric and xylo-oligomeric form) was recovered from pretreatment hydrolysate with low concentration furfural concentration indicates the OPMF was pretreated at suitable pretreatment condition. In addition, about 7.6 g xylose and 16.9 g glucose can be recovered from enzymatic hydrolysis of combined pretreatment using HCW (180°C, 20 min) and WDM_{9cycle}. Meanwhile, the xylose in the enzymatic hydrolysis residues has not been detected, due to efficient hydrolysis of hemicellulose during HCW and enzymatic digestion. Taking into consideration the highest cellulose and hemicellulose conversion and energy consumption, therefore we may conclude that combination of pretreatment using HCW (180°C for 20 min) and WDM_{9cycle} were the best method to recover the highest xylose (> 90%) and glucose (>86%), and is preferred for pretreatment of OPMF.

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371 **4. Conclusion**

372 The effect of hydrothermal and WDM pretreatment on OPMF was successfully compared
373 and discussed. Hydrothermal pretreatment alone showed an increased in degree of enzymatic
374 digestibility compared to untreated OPMF. Hemicellulose removal was more pronounced in
375 HCW and subsequent WDM treatment has promoted defibrillation of cellulose microfibril, hence
376 increased specific surface area. Furthermore, combined HCW and WDM offered shorter milling
377 cycles and lower power consumption with more than 98% of glucose yield. To the best of our
378 knowledge this is the first report of eco-friendly combined pretreatment using hydrothermal and
379 WDM to increase the enzymatic efficiency of OPMF.

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389 **References**

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Table 1 Chemical compositions of untreated and hydrothermally treated OPMF samples at varying reaction temperature and time.

*Hemicellulose dissolution, % = $(\text{Hemicellulose}_{\text{untreated}} - \text{Hemicellulose}_{\text{treated}}) / \text{Hemicellulose}_{\text{untreated}} \times 100\%$.

Table 2 Yields of hydrolyzed sugars obtained from untreated, SHS and HCW.

Table 3 Comparative analysis of yield of hydrolyzed sugars and energy consumption from SHS and HCW at different treatment severities with other studies.

*WDM= wet disk milling, SHS= superheated steam, HCW= hot compressed water, and LHWP= liquid hot water pretreatment.

FIGURE CAPTIONS

Fig. 1 Summary of experimental design employed in the present study.

Fig. 2. Combination of hydrothermal and wet disc milling pretreatments on xylose and glucose yield. Xylose (a, c) and glucose yield (b,d) were obtained from superheated steam (SHS) and hot

530 compressed water (HCW), respectively at different severity factors. 5% (w/v) substrate was
531 hydrolyzed at cellulase loading, 10 FPU/g-substrate, and incubate for 72 h at 50°C with stirring.

532

533 **Fig. 3** Mass balance of combined pretreatment using hydrothermal and wet disk milling for high
534 glucose recovery from oil palm mesocarp fiber.

535

536

Table 1 Chemical compositions of untreated and hydrothermally treated OPMF samples at varying reaction temperature and time.

Dry weight basis		Superheated steam (SHS), 60 min			Hot compressed water (HCW), 20 min		
(%)	Untreated	190°C	200°C	210°C	160°C	180°C	200°C
Cellulose	25.4 ± 0.5	34.7 ± 1.3	31.1 ± 0.6	29.8 ± 1.1	31.7 ± 0.5	34.5 ± 2.5	38.1 ± 0.5
Hemicellulose	21.2 ± 0.2	14.7± 1.5	13.5 ± 2.1	12.3 ± 0.8	21.1 ± 16.7	11.5 ± 9.5	1.1 ± 1.1
Klason lignin	24.6 ± 2.9	47.1 ± 0.2	51.0 ± 2.9	52.4 ± 0.5	29.2 ± 14.1	39.6 ± 0.5	48.8 ± 12.5

*Hemicellulose dissolution, % = (Hemicellulose_{untreated} – Hemicellulose_{treated})/ Hemicellulose_{untreated} x 100%

Table 2 Yields of hydrolyzed sugars obtained from untreated, SHS and HCW.

Pretreatments	Yields of hydrolyzed sugars	
	Glucose (%)	Xylose (%)
Untreated	16.6 ± 1.9	5.8 ± 0.5
SHS		
190°C, 60 min	16.4 ± 2.6	6.6 ± 0.2
200°C, 60 min	10.3 ± 0.6	7.9 ± 0.4
210°C, 60 min	13.1 ± 1.4	10.7 ± 0.8
HCW		
160°C, 20 min	21.4 ± 0.9	10.2 ± 0.3
180°C, 20 min	58.4 ± 3.8	14.1 ± 1.1
200°C, 20 min	62.9 ± 0.9	3.1 ± 0.1

Table 3 Comparative analysis of yield of hydrolyzed sugars and energy consumption from SHS and HCW at different treatment severities with other studies.

Sample	Yield of hydrolysed sugars,% (g/g-substrate)		Energy consumption (MJ/kg biomass)	References
	Xylose	Glucose		
OPMF WDM _{9cycle}	30.6 ± 0.5	30.2 ± 2.4	18.9	This study
SHS _{190°C, 60 min} + WDM _{9cycle}	31.3 ± 1.2	33.4 ± 3.7	10.9	This study
SHS _{200°C, 60 min} + WDM _{9cycle}	42.1 ± 1.0	47.8 ± 2.0	10.3	This study
SHS _{210°C, 60 min} + WDM _{9cycle}	24.1 ± 0.2	26.0 ± 0.9	5.1	This study
HCW _{160°C, 20 min} + WDM _{9cycle}	45.0 ± 0.9	46.3 ± 0.9	17.9	This study
HCW _{180°C, 20 min} + WDM _{9cycle}	54.3 ± 2.5	86.2 ± 1.4	14.9	This study
HCW _{200°C, 20 min} + WDM _{9cycle}	10.1 ± 0.7	91.1 ± 4.3	17.5	This study
WDM _{10cycle} -rice straw	41.5 ± 3.1	78.5 ± 5.6	5.4	Hideno et al. (2009)
WDM _{10cycle} -sugarcane bagasse	22.3 ± 1.2	31.5 ± 1.7	10.6	Silva et al. (2010)
WDM _{10cycle} -sugarcane straw	33.6 ± 0.4	56.1 ± 0.5	14.8	Silva et al. (2010)
LHWP _{180°C, 20 min} + WDM _{15cycle} -eucalyptus	88.1	91.6	-	Weiqi et al. (2013)
HCW _{135°C, 60 min} + WDM _{15cycle} -rice straw	79.0	90.0	-	Hideno et al. (2012)

*WDM= wet disk milling, SHS= superheated steam, HCW= hot compressed water, and LHWP= liquid hot water pretreatment.

Fig. 1 Schematic diagram of pretreatments on OPMF samples.

Fig. 2. Combination of hydrothermal and wet disk milling pretreatments on xylose and

glucose yield. Xylose (a, c) and glucose yield (b,d) were obtained from superheated steam (SHS) and hot compressed water (HCW), respectively at different severity factors. 5% (w/v) substrate was hydrolyzed at cellulase loading, 10 FPU/g-substrate, and incubate for 72 h at 50°C with stirring.

Fig. 3 Mass balance of combined pretreatment using hydrothermal and wet disk milling for high glucose recovery from oil palm mesocarp fiber.