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## Article

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## Research paper

# Chemical-free fractionation of palm empty fruit bunch and palm fiber by hot-compressed water technique for ethanol production



Natchanok Pangsang<sup>a</sup>, Udomsin Rattanapan<sup>a</sup>, Anusith Thanapimmetha<sup>b</sup>,  
Penjit Srinopphakhun<sup>b</sup>, Chen-Guang Liu<sup>c</sup>, Xin-Qing Zhao<sup>c</sup>, Feng-Wu Bai<sup>c</sup>,  
Chularat Sakdaronnarong<sup>a,\*</sup>

<sup>a</sup> Chemical Engineering Department, Faculty of Engineering, Mahidol University, 25/25 Putthamonthon 4 Road, Salaya, Putthamonthon, Nakorn Pathom 73170, Thailand

<sup>b</sup> Chemical Engineering Department, Faculty of Engineering, Kasetsart University, 50 Phaholyothin Road, Jatujak, Bangkok 10900, Thailand

<sup>c</sup> State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic & Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

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## ABSTRACT

The purpose of this study was to valorize oil palm empty fruit bunch (EFB) and palm fiber (PF) from palm oil mills to produce fuel ethanol. Chemical-free fractionation of EFB and PF using hot-compressed water (HCW) pretreatment at temperatures ranging from 150 to 200°C at 30 bars and reaction times for 5, 15, 25 min was applied to remove most of hemicellulose and partial lignin fractions into liquid phase. Comparison of the effects of different commercial cellulase enzyme complexes (Accellerase 1500, Cellic<sup>®</sup> Ctec2, Cellic<sup>®</sup> Htec2 and Ctec2+Htec2) on HCW pretreated materials were investigated. Finally, an inhibitory effect of using the whole slurry from HCW pretreatment of EFB and PF on enzyme hydrolysis and ethanol production by *Saccharomyces cerevisiae* TISTR5606 was studied. The finding suggested using solely solid fraction from HCW pretreatment for ethanol fermentation, otherwise some additional tannic acid, furfural and acetic acid removal steps are required to prevent the inhibition on yeast growth.

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## 1. Introduction

Currently, the demand of palm oil consumption in Asia-Pacific region has been increasing. Palm oil is a main ingredient in most of household consumer and food products e.g. detergent, soap, hair conditioner, chocolate, salad cream, etc. Apart from that, biodiesel and biojet are interesting and attractive renewable bio-fuels which have been increasingly demanded in the near future. It results in the rapid growth of oil palm industry in developing countries in South-East Asia such as Malaysia, Thailand and Indonesia. Oil palm planting would be increased by 4 to 6 million ha by the year 2030 raising oil production from 28 million tons/year to 50 million tons/year. In Thailand, refined palm oil production is estimated to produce at approximately 16 million tons per year of empty fruit bunch (EFB). Either high cost of disposal and waste treatment or burning as a low heating value solid fuel has been presently implemented in the industry (Chang, 2014; Hassan et al., 2013). Consequently, many researchers and manufacturers continuously proposed the alternatives palm oil industry's wastes

case studies for fossil fuels replacement as a substituted source of bio-fuel energy like bio-diesel, bio-ethanol (Cardona et al., 2018; Tan et al., 2016), bio-methanol (Gómez et al., 2011) and biohydrogen (Charnnok et al., 2018).

EFB and palm fiber (PF) are composed mainly of cellulose, hemicellulose and lignin. However, the structure of EFB and PF are complex and difficult to access and utilize as carbon sources to convert to bio-alcohols or biogas for microorganisms. Efficient fractionation or pretreatment process for palm waste treatment to separate carbohydrate source for biotechnological conversion has been intensively investigated. Recent research was related to chemical pretreatments of EFB to produce bioethanol as alternative biofuels e.g. aqueous ammonia pretreatment (Jung et al., 2011), peracetic acid/alkaline peroxide pretreatment (Palamae et al., 2017), phosphoric acid pretreatment (Ishola et al., 2014), bisulfite pretreatment (Tan et al., 2016), and organosolv pretreatment vs acid hydrolysis of EFB (Nurfahmi et al., 2016). Although high sugar concentration and ethanol yield were obtained from most of chemical pretreatment, the investment of wastewater pretreatment, chemical recovery system and washing or neutralization steps of hydrolysate prior to ethanol fermentation are costly and led to non-feasible process.

\* Corresponding author.

E-mail address: [chularat.sak@mahidol.ac.th](mailto:chularat.sak@mahidol.ac.th) (C. Sakdaronnarong).

Hydrothermal or hot-compressed water (HCW) pretreatment is another technique to increase the accessibility efficiency of lignocellulosic materials for bioconversion by enzyme or micro-organism. HCW was conducted at temperatures in the range of 150–260 °C for short time (5–20 min), especially water near the critical point changes solvent properties from ionic to non-ionic media, which results in dissolving organic matters into the liquid. Hemicellulose is substantially separated from the lignocellulosic material and solubilized in aqueous phase as a soluble oligosaccharide. In particular, the HCW technique also breaks down the bonding between cellulose–hemicellulose–lignin structure, resulting in better accessibility to cellulose moieties. As in hydrothermal or HCW pretreatment, the number of dissolved solids in the liquid is diluted, therefore dehydration reaction of released sugar to furans and condensation reaction of dissolved lignin are minimized. Recently, Goh et al. (2010) studied the hydrothermal pretreatment of oil palm frond (OPF) to enhance sugar production from enzyme hydrolysis. The Central Composite Design (CCD) was used to optimize the pretreatment conditions for OPF. The most suitable condition was at 10 bar at 178 °C for 11.1 min and a liquid-to-solid ratio was 9.6, from which glucose yield of 92.78% was achieved (Goh et al., 2010). Subsequently, Goh et al. (2012) studied the influence of severity factor on substrate digestibility including temperature (160–200 °C), time (45–90 min) and liquid-to-solid ratio (8–16 v/w) on OPF pretreatment using HCW. The results suggested that HCW pretreatment significantly increased glucose yield up to 83.72% when the severity was 3.31 and liquid-to-solid ratio was 8.0 compared with the glucose yield of 30.97% from untreated OPF (Goh et al., 2012). In case of EFB and PF, only little was conducted on the HCW pretreatment for ethanol production (Zakaria et al., 2015a; Akhtar et al., 2010a). Combined mechanical pretreatment using disk milling and chemical pretreatment using alkaline catalyst was investigated as a costly process.

HCW technique has several advantages including environmentally friendly, inexpensive process due to no solvent recovery system (Charnnok et al., 2018; Weerasai et al., 2018; Namchot et al., 2014). Additionally, low level of phenolic heterocyclic compounds were generated e.g. 5-hydroxymethyl furfural (HMF) and furfural that are inhibitors of microorganisms. Consequently, this study aimed to valorize PF and EFB to produce fuel-grade ethanol. Fractionation of EFB and PF by HCW system was conducted by varying crucial parameters i.e. temperature and time. Screening of commercial enzymes suitable for maximizing cellulose hydrolysis yield was carried out. Finally, the comparison study on utilization of solid HCW treated fraction and utilization of the whole slurry of EFB and PF from HCW pretreatment for ethanol production by a wild yeast strain *Saccharomyces cerevisiae* TISTR5606 was investigated.

## 2. Materials and methods

Wastes of oil palm (*Elaeis guineensis* jacq.) including EFB and PF were supplied by Chumporn Palm Oil Industry Public Co., Ltd., Thailand. Commercial enzymes were purchased accordingly Accelerase 1500 (Genencor, USA), Cellic<sup>®</sup> Ctec2 (Novozyme) and Cellic<sup>®</sup> Htec2 (Novozyme). Standard sugars (glucose, xylose, arabinose, cellobiose), furans derivations (5-Hydroxymethyl furfural (HMF) and furfural), and organic acids (formic acid and lactic acid) for High performance liquid chromatography (HPLC) were from Sigma-Aldrich. Standard acetic acid and absolute ethanol were purchased from Merck. The reagents for reducing sugar analysis including 3,5-dinitrosalicylic acid (DNS) and sodium-potassium tartrate were from Sigma-Aldrich. For xylanase and cellulase enzyme activity assay, xylan and Whatman filter paper no.1 were purchased from Sigma-Aldrich, respectively.

### 2.1. Hot-compressed water pretreatment of EFB and PF

EFB and PF from factory were sun-dried to the moisture content of 18%–20%, milled and sieved to a certain range of particle sizes (+50/–200 mesh). Prior to use, milled EFB and PF samples were dried in an oven overnight at 80 °C to the moisture content of 4.5%–5.0%. Then, 10 g of EFB or PF and 100 mL deionized water was added into a 500 mL reactor (solid: liquid ratio of 1: 10). For fractionation of EFB, the reaction was performed between 150 and 200 °C and reaction time for 5, 15, 25 min. The fractionation of PF was performed between 180 and 200 °C and reaction time for 5, 15, 25 min which was the suitable range selected from EFB pretreatment. After the reaction, solid and liquid parts were separated by vacuum filtration. Solid residue was weighed and the moisture content was measured to determine the solid recovery (dry basis) as shown in Eq. (1).

Solid recovery (%)

$$= \frac{\text{Wet treated biomass (g)} \times (1 - \text{moisture content (\%)/100})}{\text{Dry weight of EFB or PF before pretreatment (g)}} \times 100 \quad (1)$$

### 2.2. Characterization of untreated and pretreated EFB and PF

Surface morphologies of untreated and HCW pretreated EFB and PF were investigated by a scanning electron microscope (SEM) of model SU3500, Hitachi, Japan at  $\times 1000$  magnifications. The changes of functional groups of samples were analyzed using Fourier Transformed Infrared spectroscopy (FTIR), model FTIR-6800, JASCO, USA. The FTIR spectrum in transmittance mode was recorded in range of 4000–400  $\text{cm}^{-1}$  with 100 numbers of scan using ATR PRO ONE technique. Each sample was measured for triplicates and the spectra were averaged.

Crystalline structure of HCW pretreated EFB and PF were analyzed by X-ray Diffractometer (XRD), model D8 Advance: Bruker AXS, Germany using Cu radiation at wavelength 1.5406 nm, the voltage at 40 kV and the current at 30 mA. The condition used to scan powder samples was at  $2\theta$  ranging from 5° to 80° with scan speed of 0.5 degree per min. The XRD intensity at particular region of crystalline at plane 200 ( $I_{200}$ ) and amorphous moieties ( $I_{am}$  at approximately  $2\theta = 18^\circ$ ) of sample were used to calculate the crystallinity index (CrI) of the sample according to Segal-WAXS CrI method as shown in Eq. (2) (Lani et al., 2014; Segal et al., 1959) which is an empirical method to estimate crystallinity native cellulose (Table S1).

$$\text{Crystallinity index} = \frac{I_{200} - I_{am}}{I_{200}} \times 100\% \quad (2)$$

Surface area, pore size diameter and pore volume of EFB and PF pretreated samples were analyzed by Autosorb 1-AG (Quantachrome, USA) and the values were calculated based on Brunauer–Emmett–Teller (BET) method.

### 2.3. Screening of cellulase complexes for hydrolysis of HCW pretreated EFB and PF

To compare the efficiency of HCW pretreatment for fermentable sugars production, the sugar monomers released from enzyme hydrolysis of untreated and HCW treated EFB and PF were determined. The hydrolysis took place in 100-mL Erlenmeyer flask in which 0.2 g dry untreated EFB, untreated PF, treated EFB or treated PF from HCW pretreatment at 200 °C for 15 min together with 19.2 mL of 50 mM sodium acetate buffer pH 4.8 and 0.4 mL cellulase and hemicellulase enzyme complexes (~200 FPU/g dry substrate) were added. Cellulase enzyme complexes used in this study included Accelerase 1500 (Genencor,

USA) and Cellic® Ctec2 (Novozyme) while hemicellulase enzyme was Cellic® Htec2 (Novozyme). Prior to the study, activity of each crude cellulase enzyme (Accelerase 1500 and Ctec2) was determined as filter paper unit assay (FPU/mL) using Whatman filter paper No.1 as the substrate while xylanase activity of Htec2 was determined when using xylan as the standard. The reaction took place at 50 °C for 1 h and after that analysis of reducing sugar was performed according to DNS assay using glucose standard as equivalence (Miller, 1959). Activity of each enzyme was calculated according to NREL procedure (Resch et al., 2015).

The hydrolysis took place in a shaking incubator at 50 °C with the shaking speed of 150 rpm. The supernatant was collected every 24 h from 0 h to 96 h. Amount and species of fermentable sugars were identified by High performance liquid chromatography (HPLC) having glucose, xylose, arabinose, cellobiose as the standard. The best HCW pretreatment condition which gave the highest glucose yield and/or glucose recovery was selected for scaling up and used as substrate for ethanol fermentation. The glucose and xylose yields from pretreated EFB or PF were calculated based on cellulose and hemicellulose contents in pretreated EFB or PF, respectively according to Eqs. (3) and (4) given in Box 1. Glucose recovery was calculated based on initial weight of untreated EFB or PF according to Eq. (5).

Glucose recovery based on initial weight of EFB or PF (%)

$$= \frac{\text{Glucose yield (\%)} \times \text{Solid recovery (\%)}}{100} \quad (5)$$

2.4. Separate hydrolysis and ethanol fermentation (SHF) of solid treated EFB and PF compared with whole slurry hydrolysis and fermentation (solid + black liquor)

The experiments were divided into two methods: (1) fermentation of solid treated EFB and PF, and (2) fermentation of whole slurry (solid + black liquor) from EFB and PF pretreatment. For the enzymatic hydrolysis of solid treated EFB and PF, the amount of 5 g dry pretreated EFB or PF from HCW pretreatment at 200 °C for 15 min (30 bar) was enzymatically hydrolyzed in a total 100 mL solution containing the commercial cellulase (Cellic® Ctec2, Novozyme) at the loading of 100 FPU/g substrate and 0.05 M sodium citrate buffer solution (pH 5) at 50 °C for 72 h in a shaking incubator at 150 rpm. Thus, the initial concentration of solid pretreated EFB or PF was 50 g/L treated EFB or PF (5%w/v). For the enzymatic hydrolysis of the whole slurry from EFB and PF pretreatment, initial 10 g dry EFB and PF underwent HCW in 100 mL deionized water at 200 °C for 15 min at 30 bar. After HCW pretreatment, the whole slurry was used for enzymatic hydrolysis with cellulase 100 FPU/g substrate.

In case of ethanol fermentation, the inoculum preparation was conducted using two sub-cultivation of *Saccharomyces cerevisiae* TISTR 5606 using 10%v/v inoculum in YPD medium for every 24 h in a shaking incubator at 180 rpm at 30 °C to obtain the cell concentration over 10<sup>8</sup> cells/mL. For ethanol fermentation of EFB and PF hydrolysate, the experiments were carried out in a 250-mL Erlenmeyer flask with a working volume of 100 mL. Each flask was inoculated by adding 10% (v/v) of total working volume with the prepared inoculum and the experiment was conducted for two replicates. The fermentation took place at 30 °C with a shaking speed of 180 rpm for 120 h. The fermentation broth was taken every 24 h for analysis of ethanol, residual sugars and by-products during fermentation using HPLC. Ethanol yield (g/g), fermentation efficiency based on theoretical ethanol yield (%) and ethanol productivity (g/L.h) were calculated according to Eqs. (6), (7), and (8), respectively.

$$\text{Ethanol yield (g/g)} = \frac{\text{Produced ethanol (g/L)}}{\text{Initial glucose (g/L)} - \text{Residual glucose (g/L)}} \quad (6)$$

$$\text{Fermentation efficiency (\%)} = \frac{\text{Ethanol yield}}{0.511} \times 100\% \quad (7)$$

$$\text{Ethanol productivity (g/L h)} = \frac{\text{Ethanol concentration (g/L)}}{\text{Fermentation duration (h)}} \quad (8)$$

## 2.5. Analyses

### 2.5.1. Chemical composition of EFB and PF before and after hot-compressed water pretreatment

Chemical composition of dry untreated and pretreated EFB and PF samples were analyzed by Goering and Van Soest method (Goering and Soest, 1970). Cellulose, hemicellulose and lignin content (%wt) in a sample were quantified by using Eqs. (9)–(11) after determination of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and insoluble ash.

$$\text{Cellulose (\%)} = \text{ADF (\%)} - \text{ADL (\%)} \quad (9)$$

$$\text{Hemicellulose (\%)} = \text{NDF (\%)} - \text{ADF (\%)} \quad (10)$$

$$\text{Lignin (\%)} = \text{ADL (\%)} - \text{Ash (\%)} \quad (11)$$

### 2.5.2. Analysis of sugar monomers, intermediates and ethanol using high performance liquid chromatography (HPLC)

Prior to HPLC analysis, the supernatant from enzymatic hydrolysis and fermentation broth was filtered through 0.2 µm nylon filter. The amount of sugar monomers, derivatives and ethanol was measured by HPLC (Waters model Alliance 2690, USA) equipped with 300 mm × 7.8 mm Aminex HPX-87H Ion Exclusion Column (BioRad, USA) at the column temperature of 60 °C equipped with refractive index (RI) detector. The mobile phase was 0.6 mL/min of 0.005 M sulfuric acid.

## 3. Results and discussion

### 3.1. Fractionation of EFB and PF using hot-compressed water technique

Chemical composition of treated and untreated EFB samples is presented in Fig. 1(A). From the results, fractionation of EFB by HCW at 150–200 °C for 5, 15 and 25 min at 30 bar gave significant increase of cellulose content in solid treated EFB. At the milder condition between 150 °C and 160 °C of HCW pretreatment of EFB, hemicellulose was apparently degraded (up to 38.5% of hemicellulose removal at 160 °C for 25 min) and released into aqueous phase while lignin content in treated EFB was not significantly different from untreated EFB (9.3%–11.6% lignin). The results were in good agreement with previous reports on fractionation of hemicellulose from lignocellulosic biomass using hydrothermal technique from which hemicellulose were mostly released while cellulose and lignin still remained intact (Saha, 2003; Sukhbaatar et al., 2014). The main reason was because water becomes acidic or protonated to hydronium ions (H<sub>3</sub>O<sup>+</sup>) at elevated temperature and subsequently hydronium ions act as catalyst for the release of acetyl groups from hydrothermolysis into liquid phase (Garrote et al., 2001). In addition, the deacetylation of hemicellulose consequently led to generated acetic acid which acts as a catalyst for secondary hydrolysis reactions. The hydrolysis sensitive linkages which are found in lignocellulosic structure include acetyl groups on hemicelluloses (Yelle et al., 2013), glycosidic linkages in polysaccharides (Visuri et al., 2012), and ether linkages in lignin (Zhu et al., 2015). The hydrolysis of acetyl groups is of specific interest as acetic acid is produced and catalyzes secondary hydrolysis reactions (Giummarella and Lawoko, 2017). Adding homogeneous or heterogeneous acid catalyst has been revealed to decrease the temperature of hydrothermal pretreatment to extract hemicellulose into aqueous phase (Namchot et al., 2014; Sakdaronnarong et al., 2016, 2014).



$$\text{Glucose yield (\%)} = \frac{\text{Concentration of glucose } \left(\frac{\text{g}}{\text{L}}\right) * 0.9}{\text{Weight of pretreated EFB or PF before hydrolysis } \left(\frac{\text{g}}{\text{L}}\right) \times \text{cellulose content (\%)} / 100} \times 100 \quad (3)$$

$$\text{Xylose yield (\%)} = \frac{\text{Concentration of xylose } \left(\frac{\text{g}}{\text{L}}\right) * 0.88}{\text{Weight of pretreated EFB or PF before hydrolysis } \left(\frac{\text{g}}{\text{L}}\right) \times \text{hemicellulose content (\%)} / 100} \times 100 \quad (4)$$

### Box 1.

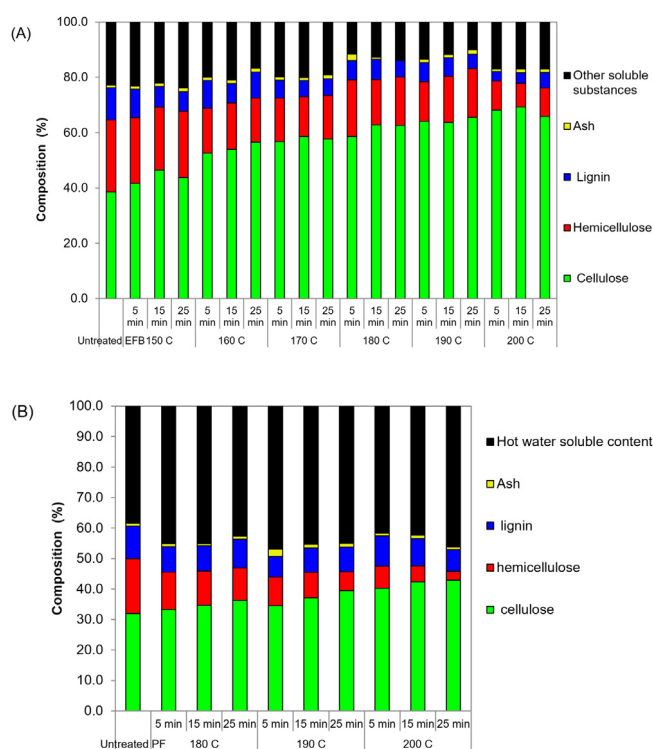
By using HCW fractionation, it was found that hemicellulose extracted was recovered as oligomers rather than polymeric sugars expressed as xylooligosaccharides in HPLC chromatogram (data not shown). At this severity, there was no by-product from hemicellulose degradation or dehydration found i.e. furfural, formic acid and acetic acid. These hemicellulose degrading products have been reported for their inhibitory effect on enzyme activity and fermentation process (Kumar et al., 2011).

When increasing HCW temperature of EFB fractionation to 190 °C and beyond, cellulose content increased substantially from 38.50% to 63–69.3% due to hemicellulose removal and partially lignin degradation. Xylooligosaccharides hydrolysis products and partial lignin degradative products i.e. coniferyl and sinapyl alcohols were found when heating to 200–220 °C for 10 min at 100 bar while cellulose molecules required higher energy to breakdown between 250–270 °C for 30–35 min at 100 bar (Takada et al., 2018). The alkaline nitrobenzene oxidation and 2-D NMR analysis indicated that lignin was depolymerized by cleaving ether-type linkages (Takada and Saka, 2015), however the condensed-type linkages were resistant to be cleaved under HCW condition and remained in EFB and PF as the insoluble residue. Similarly, in the present study, the highest cellulose content in pretreated EFB (69.27%wt), lowest lignin (3.77%wt) and hemicellulose residues (8.6%wt) were found from HCW pretreatment at 200 °C for 15 min at 30 bar.

Considering the severity of HCW pretreatment, when increasing the temperature to 190 °C and 200 °C, an enhanced acetic acid generation in black liquor was observed. When lengthen the reaction duration, by-products from the catalytic reaction were generated because acetic acid does not only act as catalyst for the cleavage of  $\beta$ , 1–4 glycosidic bond in the hemicellulose (Jiang et al., 2016; Li et al., 2014) but also catalyzes the dehydration reaction of glucose and xylose to other by-products e.g. formic acid, furfural and 5-hydroxymethyl furfural (HMF). However, an increase of reaction time more than 15 min under HCW at 200 °C apparently caused the decrease of cellulose content in pretreated EFB due to cellulose decomposition, so called autohydrolysis, to oligomers with a shorter chain length and solubilized into black liquor (Zakaria et al., 2015b).

In case of PF fractionation using HCW technique, the selected temperature range was between 180 and 200 °C according to the EFB results. Similar profiles of cellulose content enhancement as well as hemicellulose reduction were obtained from PF pretreatment relative to EFB pretreatment. The highest cellulose content from solid treated PF was 42.87%wt with the lowest lignin content at 2.90%wt and hemicellulose content at 5.2%wt from HCW pretreatment at 200 °C for 25 min at 30 bar as shown in Fig. 1(B). Compared with EFB, cellulose content from PF was 62%wt lower than that from EFB. To select the superior substrate, subsequent enzymatic hydrolysis and ethanol fermentation were subsequently studied.

From the chemical composition analysis, it is interesting to study the extraction process prior to HCW pretreatment to eliminate all extractives including alcohol–benzene, ether and hot water soluble substances which were found in PF and EFB for more than 34–44%wt (Sreekala et al., 1997). Extractives in PF

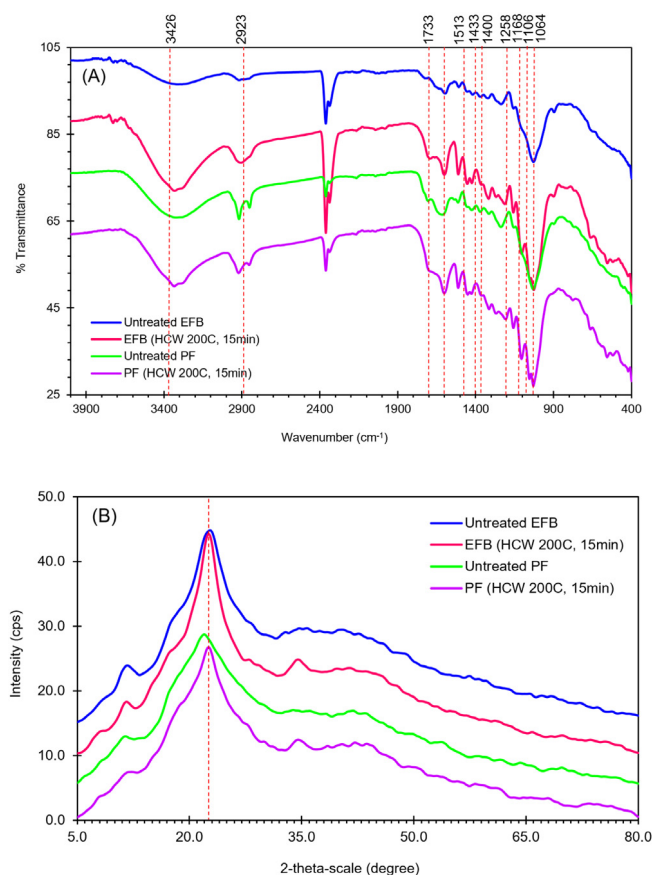


**Fig. 1.** Chemical composition of (A) solid treated EFB at 150–200 °C for 5, 15 and 25 min and (B) solid treated PF at 180–200 °C for 5, 15 and 25 min by Hot compressed water.

was considerably higher than that in EFB for about 10%. Therefore, the removal of extractives prior to pretreatment presumably enhances the efficiency of HCW pretreatment to fractionate hemicellulose and lignin which led to increased purity of cellulose in HCW treated EFB and PF.

### 3.2. Characterization of untreated and pretreated EFB and PF

As shown in Fig. 2(A), analysis of functional groups of the untreated and pretreated EFB and PF samples (200 °C for 15 min, 30 bar) was conducted by FTIR spectroscopy (ATR PRO ONE mode). The peaks at 1433 and 1513  $\text{cm}^{-1}$  attributed to aromatic structure of lignin (Sakdaronnarong et al., 2014; Sun et al., 2005; Oonkhanond et al., 2017) are prominent after HCW pretreatment relative to untreated EFB and PF. This was caused by either removal or peeling of hemicellulose surrounding the lignin–cellulose matrix confirming that HCW pretreatment insignificantly facilitates delignification of lignocellulosic biomass. Furthermore, the absorption bands assigned to hemicellulose moieties, i.e. the bands at 1258, 1400, 1733  $\text{cm}^{-1}$  (Sakdaronnarong et al., 2014; Oonkhanond et al., 2017; Peng et al., 2012) were disappeared or less intense due to the degradation or depolymerization of hemicellulose during HCW pretreatment. On the other



**Fig. 2.** (A) FTIR spectra of Untreated EFB, Untreated PF, EFB (HCW 200 °C, 15 min) and PF (HCW 200 °C, 15 min); and (B) XRD patterns of Untreated EFB, Untreated PF, EFB (HCW 200 °C, 15 min) and PF (HCW 200 °C, 15 min).

hand, intensity of cellulose was increased significantly after HCW pretreatment of EFB and PF compared to untreated samples. The band intensity at 2923 cm<sup>-1</sup> was due to the C–H stretching and the band at 3426 cm<sup>-1</sup> relates to the stretching of H-bonded OH groups. The absorption bands at 1064, 1106 and 1168 cm<sup>-1</sup> (Liu et al., 2006) were also presented sharply in HCW pretreated EFB and PF. High peak intensity at 1106 and 1064 cm<sup>-1</sup> are attributed to C–O stretching at C-3, as well as C–C stretching and C–O stretching at C-6 (Liang and Marchessault, 1959) while a shoulder at 1168 cm<sup>-1</sup> indicated the presence of C–O–C stretching at the  $\beta$ -(1→4)-glycosidic linkages (Oh et al., 2005). The FTIR spectroscopic analysis therefore presents that cellulose-rich material containing considerable amount of lignin with minimal amount of hemicellulose was obtained after HCW pretreatment at 200 °C, 30 bar for 15 min.

The XRD patterns presented in Fig. 2(B) indicated that the cellulose crystal structure of untreated EFB and PF at the plane 200 (approximately 22°) (Parshetti et al., 2013) showed less intensity compared with pretreated EFB and PF by HCW at 200 °C, 30 bar for 15 min. Accordingly, the crystallinity index (%CrI) of untreated EFB and PF was less than that of HCW pretreated sample as shown in Table 1. The results demonstrated that the CrI of EFB samples was increased from 75.06% to 78.48% for HCW pretreated EFB. Similarly, CrI of PF was increased 5.35% after pretreated. This confirmed that HCW pretreatment at 200 °C for 15 min could effectively eliminate a great amount of hemicellulose and partial amount of lignin moieties which are amorphous while cellulose which has crystalline structure remained intact. The findings were in good agreement with a previous work on the

HCW pretreatment (200 °C for 15 min) of poplar biomass from which the CrI of pretreated substrate was substantially enhanced from 60.8% to 75.6% of HCW pretreated biomass relative to untreated biomass (Lee et al., 2017). The increased CrI of pretreated substrate facilitated the efficient cellulose enzymatic hydrolysis as the lower enzyme loading and the greater glucose yield were obtained from the HCW pretreated biomass.

Characterization of physical property changes after HCW pretreatment of EFB and PF could additionally be observed by isothermal nitrogen adsorption/desorption analysis. As shown in Table 1, there was no significant difference of surface area and pore size before and after HCW pretreatment of EFB. Moreover, pore volume of HCW pretreated EFB slightly decreased which was possibly due to the collapse of pore structure caused by removal of hemicellulose. This led to destruction of biomass structure together with the evidence of shrinking, melting or fusing of lignin starting from 200 °C to 400 °C depending on their source, type, isolation method and molecular weight (Sakdaronnarong et al., 2018; Jonglertjunya et al., 2014). In contrast to EFB, HCW pretreatment of PF provided a substantial increase of pore surface area and pore volume while pore size was insignificantly changed. Therefore, the further experiment on enzymatic hydrolysis was studied to better understand the effect of characteristic changes on their hydrolyzability and fermentability of pretreated EFB and PF.

The SEM images of untreated and HCW pretreated EFB and PF at 200 °C, 30 bar for 15 min are shown in Fig. 3. As shown in Fig. 3(A) for untreated EFB, outer smooth surface due to lignin–hemicellulose layer with small circular silica body deposited was observed. After HCW pretreatment, the morphology of EFB was changed to rougher and fibrillous appearance was exposed as shown in Fig. 3(B). The EFB structure was disintegrated and was facilitated for enzyme penetration to cellulose fibrils. Similar result could be found for the chemical pretreatment of EFB or other lignocellulose at mild condition (25–35 °C), however high concentration of chemical was required (Sakdaronnarong et al., 2014; Sukiran et al., 2017). In case of PF, the structure of untreated PF shown in Fig. 3(C) was considerably different from untreated EFB since the PF was derived from pressing process to release oil from pericarp of oil palm fruit. Therefore, PF was firstly disintegrated during the palm oil processing and thus led to more rupture structure (Fig. 3(D)) compared with HCW pretreated EFB. Accordingly, substantially lower solid recovery (46% wt) of PF was obtained after HCW pretreatment as shown in Table S2.

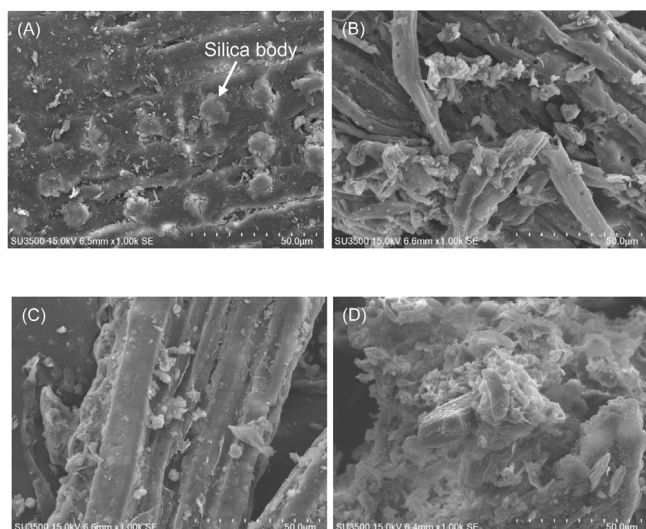
### 3.3. Enzymatic hydrolysis of EFB and PF after HCW pretreatment

To determine the effect of severity of HCW pretreatment on enzymatic hydrolyzability of solid treated EFB and PF, Accelerase 1500 was used at 200 FPU/g dry substrate at 1% substrate loading. From the results, cellobiose, glucose, xylose, arabinose and acetic acid were found in treated EFB and PF hydrolyzate (Fig. 4). For solid pretreated EFB, the highest glucose yield based on cellulose content in treated EFB was achieved at 86.8% when treated at 200 °C for 25 min. HCW pretreatment at 190 °C for 25 min gave insignificant different result of 86.3% glucose yield as shown in Fig. 4(A). The HCW temperature below 180 °C gave very low glucose yield less than 51.7% based on cellulose content in treated EFB. The reason was because HCW pretreatment at low temperature gave little effect on the destruction of EFB structure to open up the fiber for cellulase enzyme compared with HCW pretreatment at higher temperature. Similar results were found for hemicellulose extraction from bagasse which required longer time over 50 min at 170 °C of HCW condition to achieve 80% sugar concentration while the shorter time at approximately 20–25 min was required at HCW temperature of 180 °C to achieve 80%

**Table 1**

Surface area, pore size and pore volume of untreated and pretreated EFB and PF after HCW at 200 °C, 30 bar for 15 min.

| Properties                       | Untreated EFB          | Untreated PF           | Pretreated EFB<br>(HCW 200 °C, 15 min) | Pretreated PF<br>(HCW 200 °C, 15 min) |
|----------------------------------|------------------------|------------------------|--|---------------------------------------|
| Crystallinity index (%CrI)       | 75.06%                 | 66.08%                 | 78.48%                                 | 71.43%                                |
| Surface area (m <sup>2</sup> /g) | 13.36                  | 5.297                  | 11.07                                  | 18.42                                 |
| Pore size (Å)                    | 21.60                  | 20.35                  | 19.10                                  | 19.04                                 |
| Pore volume (cm <sup>3</sup> /g) | $6.289 \times 10^{-3}$ | $5.330 \times 10^{-3}$ | $2.605 \times 10^{-3}$                 | $8.581 \times 10^{-3}$                |



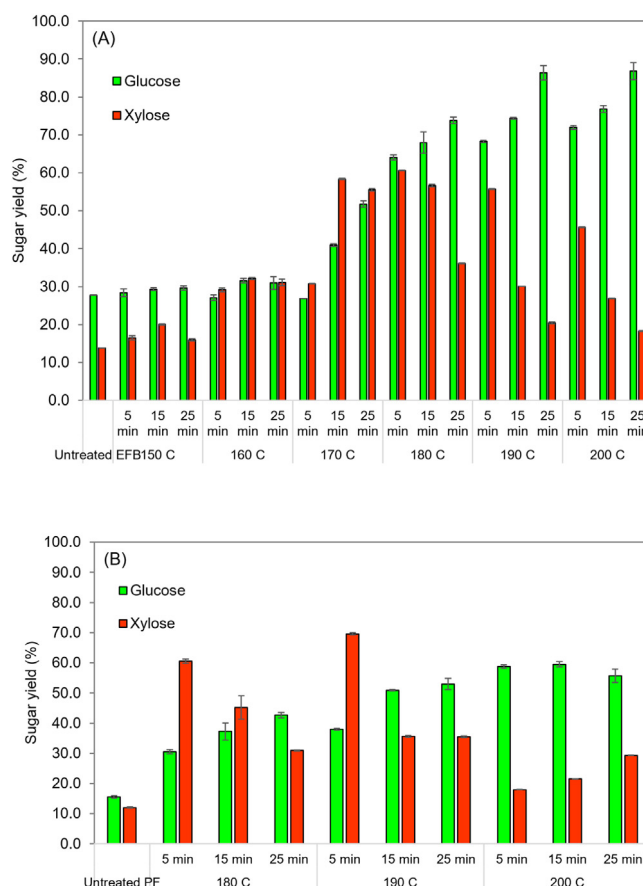
**Fig. 3.** SEM image analysis at  $\times 1000$  magnification of (A) Untreated EFB; (B) HCW EFB; (C) Untreated PF; (D) HCW PF when the HCW pretreatment was at 200 °C, 30 bar for 15 min.

sugar concentration in liquid phase (Sukhbaatar et al., 2014). At sufficient energy supply to the thermal decomposition of hemicellulose, the H-bonding starts weakening which allows auto-ionization of water into acidic hydronium ions ( $H_3O^+$ ) (Ruiz et al., 2013). Therefore, the longer pretreatment time for 25 min at 190 °C and 200 °C of pretreatment substantially increased hemicellulose removal and markedly increased glucose yield based on cellulose content in the substrate. Consequently, correlation between temperature and time strongly influences the physico-chemical characteristics of lignocellulosic material from HCW pretreatment.

Similar results were found in PF as shown in Fig. 4(B) when the HCW temperature at 200 °C for 15 min gave the highest glucose yield at 59.5% compared to 200 °C for 5 min. In contrast, xylose was found in higher amount from HCW pretreated PF at 180 and 190 °C for only 5 min and the xylose yields were 60.6 and 69.6%, respectively. An increase of pretreatment time to 15 and 25 min substantially enhanced hemicellulose removal from PF, and thus less xylose yield was obtained. At 200 °C of HCW pretreatment, almost all hemicellulose was removed which led to enhanced cellulase accessibility to cellulose moiety and increased glucose yield.

#### 3.4. Screening of commercial cellulase and xylanase enzyme complexes for EFB and PF

To enhance the polysaccharides hydrolysis, synergistic effect of glycoside hydrolases, lignin modifying enzymes (Singhania et al., 2013) and other assistive enzymes is a key success which refers to the promising hydrolysis efficiency i.e. a greater hydrolysis yield in a shorter time (Malgas et al., 2017). The synergistic consequence of two or more enzymes could be represented by



**Fig. 4.** (A) Sugars yields from enzymatic hydrolysis (50 °C for 72 h) of HCW pretreated EFB at 150–200 °C for 5, 15 and 25 min, and (B) Sugars yields from enzymatic hydrolysis (50 °C for 72 h) of HCW pretreated PF at 180–200 °C for 5, 15 and 25 min.

the degree of synergism (DS) calculated by dividing the activity of the combined enzymes by the theoretical sum of their individual activities on the same substrate. Two different patterns of synergistic effect have been categorized; (1) negative sign which means that the highest degree of synergy is observed at the early stages with the degree of synergy decreasing towards the latter stages of hydrolysis (Andersen et al., 2008), and (2) positive sign which means that the highest degree of synergy is observed during later stages (Jung et al., 2007). In the present study, apart from the performance of Accelerase 1500 and Ctec2, the synergism between Ctec2 and Htec2 was also investigated for the time-dependent sugar yield from hydrolysis of HCW pretreated EFB and PF.

From the results of hydrolysis yield, it exhibited that the combined Ctec+Htec substantially enhanced hydrolysis yield at the early stage of hydrolysis process when the substrate was HCW treated EFB and PF at 180 °C for all pretreatment times (5, 15 and 25 min), so that the DS was positive (+) at 0 to 48



h, and after that the DS was intermediate level ( $\pm$ ) until the end of hydrolysis period at 96 h. The reason was possibly due to the residual hemicellulose and undisruptive structure of HCW pretreated EFB and PF which was shielded from hydrolysis by Ctec2, therefore the synergism of Htec considerably affected to accelerate the hydrolysis rate at the early stage. Once the limited cleavage sites for the Ctec2 enzymes were accessible due to the unshielded of cellulose by hemicellulose peeling, cellulose moiety was susceptible for Ctec2 to be hydrolyzed.

However, depending on the pretreatment severity for HCW treated EFB and PF at 190 °C and 200 °C at any pretreatment time, the DS was negative (–) for the combination of Ctec2+Htec2 compared with only Ctec2 that could accelerate the hydrolysis yield at the early stage of hydrolysis process. The possible reason for this pattern is that cooperation between enzymes in the early stages of enzyme hydrolysis is the most important. As the enzymes cooperate to unravel the substrate, binding sites become more available over time, requiring a lower degree of cooperation between the enzymes and, therefore, a lower degree of synergy (Van Dyk and Pletschke, 2012). From the present work, the results suggested that solely Ctec2 was sufficient for hydrolysis of pretreated EFB to achieve 55.3% glucose yield. However, in case of PF, Htec2 slightly enhanced glucose yield based on pretreated PF as it contained more hot water soluble substances to be cleaved relative to EFB as demonstrated in compositional analysis (Fig. 1). Pretreatment is necessary to obtain glucose from EFB and PF for bioconversion to ethanol.

For the glucose and xylose yield based on cellulose and hemicellulose in treated substrate as demonstrated in Fig. 5, the synergism of Ctec+Htec gave negative effect on hydrolysis yield for the later stage at 72 h of hydrolysis process of HCW treated EFB at 200 °C for 15 min. Only Ctec2 enzyme was sufficient for hydrolysis of HCW treated EFB at 200 °C for 15 min to get 79.9% glucose yield based on cellulose content while Accellerase 1500 gave only 58.0% glucose yield from the same substrate. This was mainly because the significantly higher  $\beta$ -glucosidase activity at about 435 CBU/mL of Ctec2 when compared with 385 CBU/mL of Accellerase 1500 although the total cellulase activities from these two types of enzymes were similar (258–274 FPU/mL).  $\beta$ -glucosidase completes the final step of hydrolysis by converting the cellobiose (an intermediate product of cellulose hydrolysis) to glucose, hence it is the rate-limiting enzyme. Thus during the hydrolysis process, if  $\beta$ -glucosidase is not sufficient or inactive, cellobiose gets accumulated. However, once glucose is accumulated in the medium, it also causes feedback inhibition which together exhibits inhibiting effect on the enzymatic hydrolysis of biomass (Singhania et al., 2013). Similar results were found in PF when hydrolysis yield of HCW treated PF at 200 °C for 15 min gave the insignificantly different result for only Ctec2 (100% xylose yield based on hemicellulose content and 95.3% glucose yield based on cellulose content) and combination of Ctec+Htec (100% xylose yield and 100% glucose yield) as shown in Fig. 5.

Although, glucose and xylose yields based on carbohydrate content in HCW pretreated PF were considerably higher than pretreated EFB, solid recovery after pretreatment of EFB was 60.4% which was prominently higher than that of pretreated PF (46%wt). Therefore, glucose recovery from native EFB (48.3%wt) was significantly higher than that from native PF (46%wt) as shown in Table S2.

### 3.5. Separate hydrolysis and ethanol fermentation (SHF)

#### 3.5.1. Hydrolysis of solid treated EFB and PF compared with whole slurry hydrolysis and fermentation (solid + black liquor)

From the previous experiment, HCW pretreatment at 200 °C for 15 min at 30 bar was selected as it provided high glucose yield

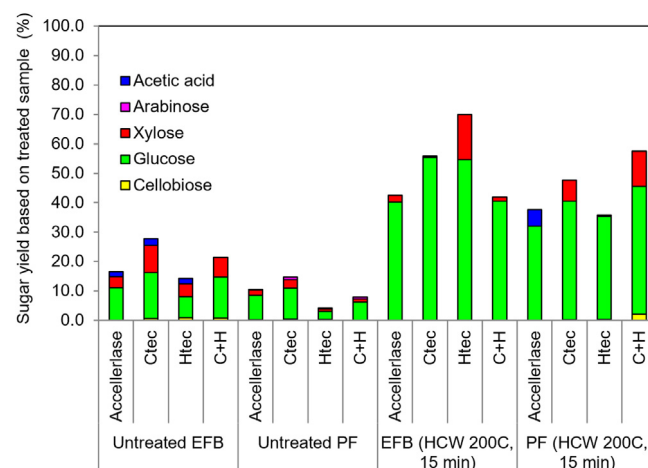


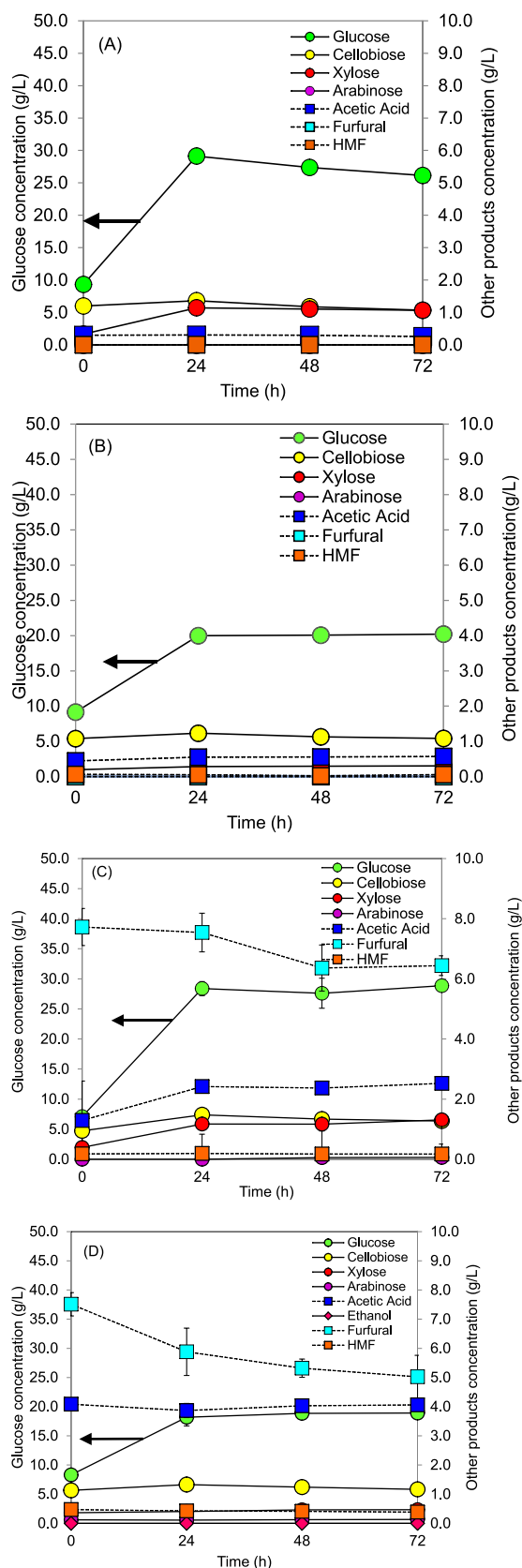
Fig. 5. Sugars yields based on dry treated sample from enzymatic hydrolysis by Accellerase 1500, Cellic® Ctec2, Cellic® Htec2 and Cellic® Ctec2 + Cellic® Htec2 at 50 °C for 72 h of untreated EFB and PF compared with HCW pretreated EFB and PF at 200 °C for 15 min.

based on cellulose content in treated EFB and PF of 76.8% glucose yield (Fig. 4(A)) and 59.5% glucose yield (Fig. 4(B)), respectively when using Accellerase 1500 (200 FPU/g) as cellulase enzyme for hydrolysis. In case of cellulose hydrolysis using Ctec2 (100 FPU/g) as shown in Fig. 5, 79.9% and 95.3% glucose yield based on cellulose content were achieved from HCW pretreated EFB and PF, respectively. Thus, Ctec2 was selected for hydrolysis of solid and solid+black liquor from HCW pretreatment of EFB and PF to prepare sugar monomers for further ethanol fermentation.

From the hydrolysis results of solid fraction, it was found that at the initial HCW treated EFB of 50 g/L (dry basis) the glucose released into hydrolysate was 29.13 g/L at only 24 h of hydrolysis corresponding to 84.1% glucose yield based on cellulose content in treated EFB (69.3% cellulose content) as shown in Table S3 and Fig. 6(A)). Relatively lower hydrolysis yield of treated EFB from the previous enzyme screening experiment was mainly due to the increase of substrate loading from 1% to 5%wt in the present experiment. Moreover, the enzyme loading was reduced to 100 FPU/g dry substrate that caused significant reduction of hydrolysis yield of HCW pretreated EFB. Similar result was observed from HCW pretreated PF and 19.98 g/L glucose concentration was obtained at only 24 h of hydrolysis from initial 50 g/L dry treated PF corresponding to 94.3% glucose yield based on cellulose content in treated PF (42.4% cellulose content) as demonstrated in Table S3 and Fig. 6(B)). An increase of hydrolysis time from 24 h to 72 h did not give any enhancement of hydrolysis yield. This was mainly due to sufficient disruption of EFB and PF structure and complete removal of hemicellulose shield from the structure at HCW pretreatment at 200 °C for 15 min at 30 bar. Thus, xylose content from hydrolysis was less than 0.5 g/L. However, cellobiose concentration from both HCW treated EFB and PF was approximately 1 g/L, therefore additional  $\beta$ -glucosidase may enhance the efficiency of cellobiose hydrolysis to glucose.

In case of hydrolysis of the whole slurry from HCW pretreatment of EFB and PF (Solid+Black liquor), slightly lower glucose was released during hydrolysis. At 24 h of hydrolysis, 28.4 g/L and 18.2 g/L glucose was determined from the hydrolysis of the whole slurry of HCW treated EFB and PF, respectively as illustrated in Fig. 6(C) and (D). It was noted that at this severity of pretreatment (200 °C for 15 min), only small amount of xylose was detected in the system. In the other hand, furfural was found in substantial amount at 7.7 g/L and 7.2 g/L from the whole slurry of EFB and PF, respectively. This was caused by xylose dehydration reaction





**Fig. 6.** Concentration of sugar monomers and derivatives from enzymatic hydrolysis at 50 °C for 0, 24, 48 and 72 h of (A) solid treated EFB, (B) solid treated PF, (C) whole slurry of EFB pretreatment, and (D) whole slurry of PF pretreatment.

to generate furfural. The most typical reaction route of xylose conversion to furfural was proposed by Dunlop reporting the certain intermediates generated further converted to furfural at elevated temperature (Chen et al., 2015).

Moreover, the presence of acetic acid in a great amount of 2.5 g/L and 4.1 g/L was detected in the black liquor from HCW pretreatment of EFB and PF at 200 °C for 15 min, respectively (Fig. 6(C) and (D)). It has been reported that organic acids formed during hydrothermal pretreatment e.g. formic acid and acetic acid could considerably catalyze the dehydration reaction of xylose to furfural (Chen et al., 2015; Lamminpää et al., 2015). It was possibly because the cations either play some role or stimulate the isomerization of xylose to lyxose or xylulose (Rasmussen et al., 2014). The selectivity and xylose conversion to furfural depends on the different reaction conditions, types of acid catalysts and the presence of other lignocellulosic derived substances such as glucose, arabinose, lignin, phenolics, etc. (Danon et al., 2014).

HCW pretreatment at temperature higher than 160 °C causes lignin to melt and lignin redeposition as micron-sized beads or globular granules on cellulose fibers during cooling process (Wang et al., 2018; Li et al., 2014b). This increases both exposed hydrophobic surface area of lignin and the protein adsorption resulting in significant loss of enzyme activities. For the presence of lignin in black liquor, it possibly provides adverse effect on enzyme adsorption and reduces the availability of cellulase binding sites toward the cellulose molecules. A previous study on different inhibition models for enzyme activity from different microorganisms after incubation in black liquor from liquid hot water (LWH) pretreatment when adding residual lignin was proposed (Zanchetta et al., 2018). This study revealed that endoglucanase activity from *Trichoderma reesei* decreased to 45, 26 and 14% of its initial activity after 1.5, 24 and 48 h of incubation when 50 mg protein/g dry lignin was used. Complete inhibition of both exoglucanase and  $\beta$ -glucosidase activities were found for cultivation of *Aspergillus niger*. Moreover, Ximenes and co-workers have demonstrated that phenolics inhibit  $\beta$ -glucosidase from *Trichoderma reesei* about twice as much as that from *A. niger* (Ximenes et al., 2011). Nevertheless, lignin has been reported to provide advantages on pH balance of black liquor during hydrothermal pretreatment and facilitate to impair furfural selectivity in the presence of formic acid (HCOOH) whereas the selectivity in sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was slightly better when lignin was added to the solution. Hence, lignin seems to have two different effects on furfural formation: (1) an acid neutralization capacity, and (2) inhibition on xylose dehydration to furfural (Lamminpää et al., 2015).

Consequently, the presence of lignin in black liquor for appropriate amount could cause beneficial effect on utilization of the whole slurry of HCW pretreatment of lignocellulose for ethanol fermentation as furfural from xylose conversion is one of the strong inhibitors on *Saccharomyces cerevisiae* growth. Therefore, an additional step after HCW pretreatment to remove lignin shield from cellulose fibers e.g. alkaline soaking at ambient or moderate temperature (Wang et al., 2018) is a promising method to enhance the hydrolysis yield and eliminate the inhibition of lignin on enzyme hydrolysis and further ethanol fermentation.

### 3.5.2. Ethanol fermentation of solid HCW treated EFB and PF compared with whole slurry hydrolysis and fermentation (solid + black liquor)

For the ethanol fermentation of solid treated EFB and PF from HCW at 200 °C, 30 bar for 15 min, small loss of glucose was found when hydrolysate from the hydrolysis stage was sterile at 121 °C for 15 min before inoculation of *S. cerevisiae*. All concentrations of substrate, product and intermediates were demonstrated in Table S3. Based on 50 g/L dry solid treated EFB and PF, initial glucose

concentration of hydrolysate from HCW treated EFB and PF were 24.85 g/L and 18.12 g/L as shown in Fig. 7(A) and (B), respectively. Ethanol was produced from 24 h of cultivation and reached the maximum concentration at 72 h for EFB hydrolysate and 48 h for PF hydrolysate that was possibly due to the lower glucose concentration in PF hydrolysate compared with EFB hydrolysate. Maximum ethanol concentration were 9.23 g/L and 7.28 g/L from EFB and PF hydrolysate at 48 h and 24 h as shown in Fig. 7(A) and (B), respectively. These corresponded to 0.37 g ethanol/g glucose or 72.41% theoretical ethanol yield for EFB hydrolysate from solid treated EFB while 0.40 g ethanol/g glucose or 78.62% theoretical ethanol yield was achieved from PF hydrolysate. However, due to the shorter time required for ethanol fermentation of PF hydrolysate, the greater ethanol productivity of 0.15 g/L·h was obtained from PF hydrolysate at 24 h of fermentation when compared with 0.13 g/L·h from EFB hydrolysate at 48 h of fermentation. The ethanol produced based on dry raw biomass were 0.11 g ethanol/g dry EFB and 0.07 g ethanol/g dry PF as demonstrated in Table 2. For other constituents e.g. cellobiose, xylose, arabinose, acetic acid, furfural and HMF, the concentration lower than 1.0 g/L were detected, and thus no effect of these substances on ethanol yield was observed.

Comparison of ethanol fermentation results from lignocellulosic biomass between other previous works and the present research was demonstrated in Table 2. The ethanol yield (g ethanol/g dry material) and productivity (g/L·h) of the present work were comparative to ethanol production from pretreated EFB and PF from other works. Nevertheless, the lower initial solid loading was utilized relative to other works and thus provided lower final ethanol concentration (Boonsawang et al., 2012; Park et al., 2013; Kim and Kim, 2013). It has been reported that an increase of initial sugar concentration or solid loading substantially influenced the maximum ethanol yield due to an enhancement of metabolic flux which led to an increased ethanol generation in the pathway of *S. cerevisiae* (Ghorbani et al., 2011; Meethit et al., 2016). Accordingly, ethanol yield and productivity were possibly improved by enhancement of initial solid loading of pretreated EFB and PF. Another method was to optimize the bioprocessing technique such as redox control or feeding manipulation e.g. fed-batch, repeated batch or pre-hydrolysis with simultaneous saccharification and fermentation of pretreated lignocellulosic substrates (Boonsawang et al., 2012; Park et al., 2013; Watanabe et al., 2012).

In contrast, ethanol fermentation when using the whole slurry (Solid+Black liquor) from HCW pretreatment of EFB and PF showed a complete inhibition on *S. cerevisiae* growth indicated by a steady cell number during fermentation as shown in Figure S1. The reason was apparently owing to the high concentration of acetic acid and furfural from xylose condensation reaction. It is known that during HCW pretreatment O-acetyl and uronic acid substitution in hemicellulose are cleaved to generate organic acid, mostly acetic acid (Mosier et al., 2005). Crucial factors that enhance acetic acid generation during HCW pretreatment are the high ratio of solid-to-liquid (high solid loadings), and an increase of pretreatment temperature and duration (Cardona et al., 2018). Furfural and HMF were also observed in the liquid fractions from HCW pretreatment. At temperature of 160 °C lower amounts of these compounds were observed, but at 180 °C, 190 °C and 200 °C the formation of these compounds was favored. On the other hand, furfural and HMF formation is also associated with an increase in the acetic acid concentration in the liquid fraction. Thus, high acetic acid concentration significantly induced the catalytic formation of furfural and HMF.

For the present study, concentration of acetic acid (2.55–4.01 g/L at 5%wt solid loading or 51–80.2 mg/g treated substrate for EFB and PF slurry respectively) and furfural (3.35–4.27 g/L

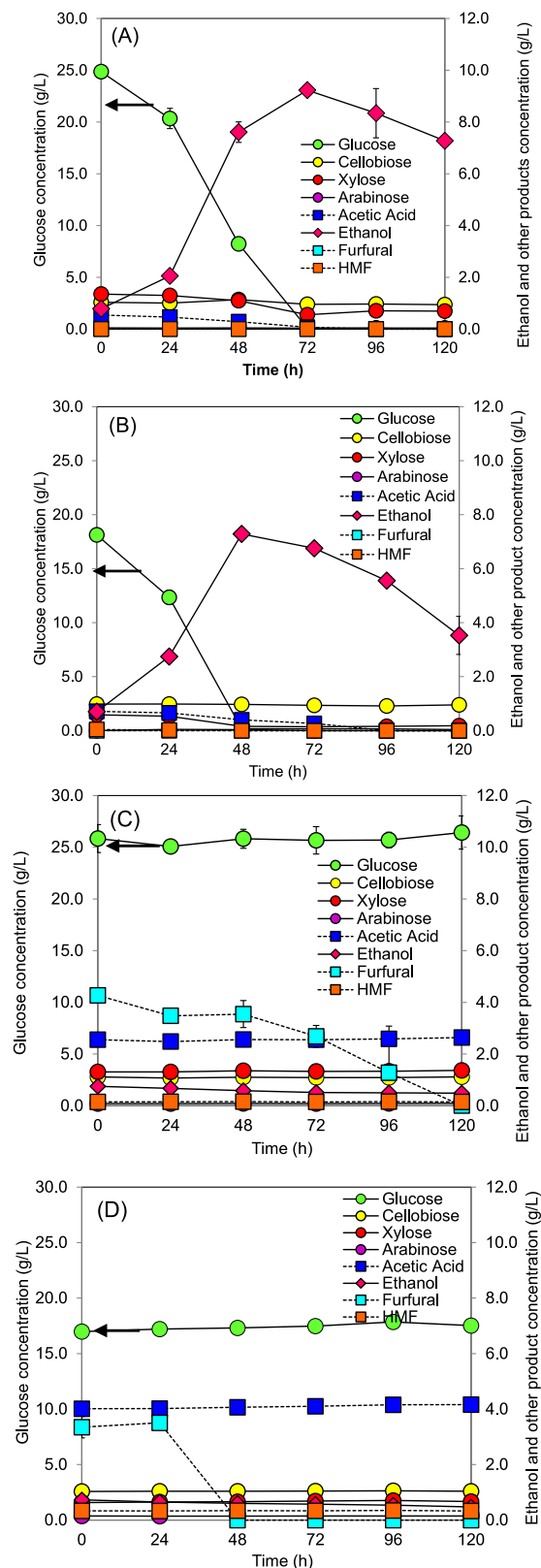


Fig. 7. Concentration of sugar monomers, derivatives and ethanol from *S. cerevisiae* fermentation at 30 °C for 120 h of (A) solid treated EFB, (B) solid treated PF, (C) whole slurry of EFB pretreatment, and (D) whole slurry of PF pretreatment.

at 5%wt solid loadings or 57–85.4 mg/g treated substrate for EFB and PF slurry respectively) were determined. These values

**Table 2**  
Ethanol yield and productivity from lignocellulosic substrates.

| Raw material<br>(Pretreatment method)                  | Strain                            | Fermentation<br>process                  | Initial substrate<br>concentration (g/L) | Ethanol<br>concentration<br>(g/L) | Productivity<br>(g/L h) | Ethanol<br>yield (g<br>EtOH/g dry<br>substrate) | Ref.                       |
|--|-----------------------------------|--|--|-----------------------------------|-------------------------|---|----------------------------|
| Rice straw (Calcium hydroxide treated)                 | <i>S. cerevisiae</i><br>NBRC0224  | Immobilized repeated-batch SSF (30 °C)   | 200 g/L rice straw                       | 38                                | 1.58                    | 0.19  | (Watanabe et al., 2012)    |
| Rice straw (Aqueous ammonia soaked)                    | <i>S. cerevisiae</i><br>D5A       | SSF (38 °C)                              | 100 g/L rice straw (30 g/L glucan)       | 12.7                              | 0.1                     | 0.13  | (Ko et al., 2009)          |
| Barley straw (Ammonia fiber expansion, AFEX)           | <i>K. Marxianus</i><br>CHY1612    | SSF (35 °C)                              | 160 g/L barley straw (35.1%wt glucan)    | 40.2                              | 1.7                     | 0.14  | (Kang et al., 2012)        |
| Switchgrass (Thermohydrolysis)                         | <i>K. Marxianus</i><br>IMB3       | SSF (45 °C)                              | 120 g/L switchgrass                      | 32                                | 0.44                    | 0.27  | (Pessani et al., 2011)     |
| Corn stover (AFEX)                                     | <i>S. cerevisiae</i><br>424A      | SHF (30 °C)                              | 60 g/L glucan loading                    | 40                                | 1.7                     | 0.19  | (Lau and Dale, 2009)       |
| Corn stover (AFEX)                                     | <i>Zymomonas mobilis</i> AX101    | SSF (37 °C)                              | 180 g/L corn stover (60 g/L glucan)      | 32                                | 0.23                    | 0.18  | (Lau et al., 2010)         |
| EFB (Thermal-Sodium hydroxide treated)                 | <i>S. cerevisiae</i><br>L2524a    | Fed-batch SSF (30 °C)                    | 300 g/L EFB                              | 60.3                              | 2.4 <sup>a</sup>        | 0.20  | (Park et al., 2013)        |
| EFB (Sequential acid/alkaline pretreated)              | <i>S. cerevisiae</i><br>W303-1A   | SHF (30 °C)                              | 100 g/L pretreated EFB                   | 37.8                              | 0.63                    | 0.189 <sup>b</sup>                              | (Kim and Kim, 2013)        |
| PF (NaOH pretreated)                                   | <i>S. cerevisiae</i><br>TISTR5596 | Batch SSF with prehydrolysis (35 °C)     | 100 g/L pretreated PF                    | 8.65                              | 0.09                    | 0.088 <sup>c</sup>                              | (Boon-sawang et al., 2012) |
| PF (NaOH pretreated)                                   | <i>S. cerevisiae</i><br>TISTR5596 | Fed-batch SSF with prehydrolysis (35 °C) | 150 g/L pretreated PF                    | 12.1                              | 0.13                    | 0.080 <sup>c</sup>                              | (Boon-sawang et al., 2012) |
| EFB (Hot compressed water, HCW 200 °C, 15 min, 30 bar) | <i>S. cerevisiae</i><br>TISTR5606 | SHF (30 °C)                              | 50 g/L pretreated EFB                    | 9.23                              | 0.13 <sup>d</sup>       | 0.11  | This study                 |
| PF (Hot compressed water, HCW 200 °C, 15 min, 30 bar)  | <i>S. cerevisiae</i><br>TISTR5606 | SSF (30 °C)                              | 50 g/L pretreated PF                     | 7.29                              | 0.15 <sup>e</sup>       | 0.07  | This study                 |

<sup>a</sup>Based on ethanol concentration of 40 g/L for industrial production.

<sup>b</sup>Based on 50% solid recovery from sequential acid/alkaline pretreatment of EFB.

<sup>c</sup>Equivalent to 0.163 g ethanol/g cellulose when cellulose content in raw PF was 54%wt.

<sup>d</sup>Ethanol productivity at 72 h of fermentation.

<sup>e</sup>Ethanol productivity at 48 h of fermentation.

were in the same range of liquid hot water pretreatment of palm empty fruit bunch from a previous work (Cardona et al., 2018). Apart from acetic acid and furfural, it has been reported that tannic acid which is rich in palm fiber could be generated during liquid hot water pretreatment at elevated temperature between 150 and 240 °C. Tannic acid showed the greatest inhibition on cellulase enzyme from *Acromonium* sp. while the presence of xylo-oligosaccharide and oil palm mesocarp fiber pretreated liquid gave inhibitory effect on cellulase in respective degree (Zakaria et al., 2016). Nevertheless, in the present study no inhibition of HCW pretreated liquid on hydrolysis efficiency of Ctec2 was obviously detected (Fig. 6(C) and (D)). Only inhibition on yeast growth was observed when the liquid from HCW pretreatment was used as the medium as shown in Figure S1(A) and S1(B) as well as the constant of glucose concentration overall fermentation period as demonstrated in Fig. 7(C) and (D). To utilize the black liquor from HCW pretreatment as the medium for the whole slurry ethanol fermentation, the detoxification process was necessary. Adsorption of tannic acid, acetic acid and furfural from black liquor from HCW was successfully performed by using activated carbon at least 5%wt (Zakaria et al., 2016).

## Conclusion

Chemical-free hot-compressed water (HCW) pretreatment of oil palm empty fruit bunch (EFB) and palm fiber (PF) significantly increased the hydrolysable sugars yield by more 200% than untreated substrates under the optimal condition at 200 °C, 30 bar for 15 min. After Ctec2 hydrolysis (100 FPU/g dry substrate) at 50 °C for 72 h, ethanol fermentation of hydrolysate from solid HCW pretreated EFB and PF yielded 0.37 g ethanol/g glucose and

0.40 g ethanol/g glucose, respectively. However, utilization of the whole slurry from HCW pretreatment was not suitable for ethanol fermentation due to inhibitors that hinder *Saccharomyces cerevisiae* TISTR5606 growth. Detoxification process or separation of cellulose-rich material are promising alternatives for efficient utilization of lignocellulose as carbon source for further biochemical conversion.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.egy.2019.02.008>.

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