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Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars

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ABSTRACT

Lignocellulose into fuel ethanol is the most feasible conversion route strategy in terms of sustainability. Oil palm empty fruit bunch (EFB) generated from palm oil production is a huge source of cellulosic material and represents a cheap renewable feedstock which awaits further commercial exploitation. The purpose of this study was to investigate the feasibility of using steam at 0.28 MPa and 140 °C generated from the palm oil mill boiler as a pretreatment to enhance the digestibility of EFB for sugars production. The effects of steam pretreatment or autohydrolysis on chemical composition changes, polysaccharide conversion, sugar production and morphology alterations of four different types of EFB namely fresh EFB (EFB1), sterilized EFB (EFB2), shredded EFB (EFB3) and ground EFB (EFB4) were evaluated. In this study, the effects of steam pretreatment showed major alterations in the morphology of EFB as observed under the scanning electron microscope. Steam pretreated EFB2 was found to have the highest total conversion of 30% to sugars with 209 g kg^{-1} EFB. This production was 10.5 fold higher than for EFB1 and 1.6 fold and 1.7 fold higher than EFB3 and EFB4, respectively. The results suggested that pretreatment of EFB by autohydrolysis using steam from the mill boiler could be considered as being a suitable pretreatment process for the production of sugars. These sugars can be utilized as potential substrates for the production of various products such as fuel ethanol.

1. Introduction

Currently more than 46 900 km^2 of oil palm are cultivated in Malaysia, the world's largest exporter of palm oil [1]. As one of the biggest exporters of palm oil and palm oil products, the palm oil industry in Malaysia generates huge quantities of biomass in the form of oil palm empty fruit bunch (EFB), oil palm shell (OPS) and oil palm fibers (OPF). The potentials of these biomasses are yet to be exploited. Out of these biomasses, EFB generated during the processing of palm oil, can be considered as a primary feedstock for the production of sugars which can be further used as carbon source for ethanol production by yeast. Ethanol production from biomass consists of four basic steps namely pretreatment, hydrolysis, fermentation and distillation. Pretreatment is the crucial step in which the biomass can be broken down into sugars through enzymatic hydrolysis enhancing the yield of saccharification.

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Investigations on the potential utilization of EFB as a substrate for ethanol production require detailed knowledge of pretreatment. There are variations in major components of EFB such as cellulose, hemicellulose and lignin, as reported by others [2–4]. The structure of EFB needs to be examined to facilitate optimal utilization of this bioresource for biofuel production. The major obstacles for sugar production are the recalcitrant nature of the raw biomass and therefore it required an additional pretreatment step to facilitate the enzymatic hydrolysis for the enhancement of sugar yield.

Steam power has the potential to degrade (thereby pretreat) the complex structure of lignocellulosic biomass. In steam pretreatment, the biomass is simultaneously treated with high pressure and high temperature steam of 140 °C–260 °C, for a few minutes to several minutes. Steam pretreatment has been reported to be efficient in partially hydrolyzing hemicelluloses, modifying the lignin, increasing access to surface area, decreasing the crystallinity of cellulose and its degree of polymerization [5]. Steam or moist heat pretreatment on lignocellulosic materials has been used by many researchers for ethanol production [6–9].

Steam pretreatment of EFB for ethanol production could be the most economical option to be implemented in the palm oil mill. During oil palm processing, steam is continuously being generated in the mill for electricity generation and for sterilizing the fruits. The boilers produce superheated steam which is used to generate electricity through a turbine generator. Low-pressure steam (140 °C, 0.28 MPa) is used for heating purposes in the factory. Every year, palm oil mills produce 50 Tg of OPS and OPF; only 60% of which are used as solid fuel for steam boilers [10]. This amount is sufficient to support the application of steam for the pretreatment of EFB in the palm oil mill. Overall, this pretreatment is attractive to be practiced in the palm oil mill as it includes renewable resources (water, OPS and OPF) and all these could be considered as inexpensive resources which are readily available in the mill.

Biomass pretreatment for ethanol production based on enzymatic hydrolysis has been described as the second most expensive unit cost [11]. Therefore, in this study a cost-effective pretreatment method strategy had been developed using the excess steam from the mill and applied to the different types of EFB (fresh EFB, sterilized EFB, shredded EFB and ground EFB) as an autohydrolysis steam pretreatment. Autohydrolysis is the process of converting lignocelluloses into sugars by exposure to high temperature steam with no addition of external catalysts. Chemical composition, sugar concentration and structural changes were determined in order to assess the effects of steam pretreatment on the different types of EFB biomass.

2. Materials and methods

2.1. *Biomass collection and preparation*

Four different EFB namely fresh EFB (EFB1), sterilized EFB (EFB2), shredded EFB (EFB3) and ground EFB (EFB4) were used in this experiment.

2.1.1. *Fresh EFB (EFB1) and sterilized EFB (EFB2)*

EFB1 and EFB2 were obtained from the FELDA Serting Hilir Palm Oil Mill, Negeri Sembilan, Malaysia. The standard grade of oil palm fruits for production of palm oil were collected from oil palm plantations (N2°48′, E102°22′) borne on bunches termed as fresh fruit bunch (FFB) which varied in weight from 10 kg to 40 kg. The FFB for palm oil processing was harvested daily in the plantations from trees aged 30 months of field planting which continue to be productive for the next 30 years. The standard quality achieved is initially dependent on the quality of the bunches arriving at the mill. For ensuring good quality of oil, the harvested bunches were loaded into lorries and rapidly transported to the factory before being transferred to sterilizer cages. In this experiment, EFB1 was an expert selection from matured quality FFB after manual cutting and threshing with chopper to detach the fruits from the bunch. Usually, the EFB constitutes about 20%–25% of FFB [12]. The EFB2 samples were obtained from the palm oil mill after the FFB was steam-sterilized with saturated steam (140 °C, 0.28 MPa) at flow rate of 5219 kg h^{-1} for 90 min using cylindrical horizontal autoclaves of approximately 1.83 m (diameter) to 3.05 m (length) with up to 3.5 Mg FFB capacity as the first step in the sequence of the processes to extract the oil [4]. There was an estimated 50 m distance from the steam pretreatment equipment to the output EFB within the factory. The sterilized EFB (EFB2) after being mechanically threshed to loosen the fruits was directly used as EFB2 starting samples. EFB1 and EFB2 were manually cut into 2.5 cm pieces, 0.25 kg each and stored in sealed plastic bags for immediate use in the pretreatment. The initial moisture content (MC) of all samples was measured.

2.1.2. *Shredded EFB (EFB3) and ground EFB (EFB4)*

Shredded EFB was provided by the Seri Ulu Langat Palm Oil Mill, Selangor, available from plantations (N2°51′, E101°39′) nearest to the palm oil mill. The freshly collected sample on 28 January 2008 was fibrous and wet. To prevent fungal contamination, it was soaked in detergent overnight before being washed and rinsed with water to remove the dirt and oil. The loose fibrous materials were then air-dried and stored at 4 °C before use. The EFB3 was clean shredded EFB with an average length of 5 cm. EFB4 was prepared by grinding EFB3 samples to average particle sizes of 2 mm. There were no visible signs of microbial contamination during the preparation of EFB3 and EFB4 samples and the handling of the biomass process for steam pretreatment. Samples of EFB3 and EFB4 were weighed, 0.1 kg each and stored in sealed plastic bags for use in the pretreatment. The initial moisture content (MC) of each sample was measured.

2.2. *Steam pretreatment (autohydrolysis)*

Steam pretreatment of EFB was carried out in a batch equipment fitted with a high pressure container, steam line from the boiler in the palm oil mill, valve, pressure and temperature gauge and blow down line to stand an operating pressure/temperature of 0.28 MPa/140 °C at FELDA Serting Hilir Palm Oil Mill as illustrated in Fig. 1. Steam was applied to 0.25 kg EFB1, 0.25 kg EFB2, 0.1 kg EFB3 and 0.1 kg EFB4 with

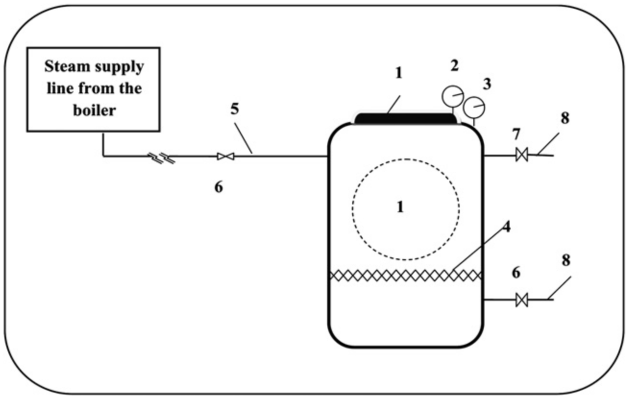

Fig. 1 – Schematic of the steam pretreatment unit used in this study. (1) feeding door; (2) temperature gauge; (3) pressure gauge; (4) sample holder; (5) steam supply line; (6) valve; (7) safety valve; (8) exhaust (blow down line).

initial moisture content of 697 g kg^{-1} EFB1, 679 g kg^{-1} EFB2, 130 g kg^{-1} EFB3 and 62 g kg^{-1} EFB4, respectively. The samples were treated in batches for (15, 30, 45 and 60) minutes. After pretreatment, the moisture contents of the samples were again measured.

2.3. *Enzymatic saccharification assessments*

Celluclast 1.5-L containing 117.6 FPU mL^{-1} of total cellulase was used for enzymatic saccharification. One FPU is defined as the enzyme amount that releases 1 μmol of glucose from Whatman no. 1 filter paper in 1 min. Enzymatic saccharification was carried out with 5% (w/v) substrate concentration in 20 cm^3 mixture of 50 mol m^{-3} sodium acetate buffer pH5 with cellulase loading of 25 FPU g^{-1} substrate in 50 mL shake flasks incubated at 40 °C and agitated at 1.67 Hz. Total reaction time was 72 h and samples were withdrawn after (0, 6, 24, 48 and 72) h of enzymatic saccharification. The results were compared by oneway analysis of variance (ANOVA) and Turkey multiple range test to find the differences between means at the 5% significance level using SPSS Statistics 17.

2.4. *Analytical methods*

The cellulose, hemicellulose and lignin content were determined by using the standard methods described in the AOAC protocol [13]. The surface morphology, cross sectional and longitudinal views of raw and pretreated fibers were examined by scanning electron microscopy, Philips model ESEM. Dried samples were mounted on the stub and were gold-coated prior to viewing under the microscope. Total reducing sugars were determined using dinitrosalicylic acid (DNS) [14]. The monosaccharides released through enzymatic saccharification were determined by a Shimadzu high-performance liquid chromatography (HPLC) using Rezex RCM-Monosaccharide 00H-0130-K0 column at 85 °C with a gradient pump and degasser and with a different refractometric detector. The mobile phase used was distilled water at a flow rate of 0.6 ml min^{-1}.

2.5. *Mass balance calculation for the steam pretreatment operation*

Mass balance calculation was doned based on dried weight of samples before and after pretreatment. The moisture content of each sample was determined by weighing the sample before and after drying at 105 °C until constant weight. The total solid content of a biomass sample is the amount of solid remaining after water has been removed by heating the sample. The final dry weight of a sample was then multiplied with the percentage of the chemical composition to obtain the actual mass involved in the pretreatment process.

3. Results and discussion

3.1. *Effects of autohydrolysis on chemical composition of different types EFB*

Autohydrolysis of biomass with steam at high temperature and pressure was catalyzed by the organic acid formed from the biomass component during the hydrolysis. Table 1 shows the main components of raw and EFB pretreated by autohydrolysis with a rapid steam from the palm oil mill's boiler. Overall, different types of EFB had different percentages of chemical composition. The values however, were in the range as reported by other researchers [3,4,15,16]. Generally, all pretreated (autohydrolysed) samples had higher percentages of cellulose and lower percentages of hemicellulose and lignin from raw samples after 15 min–60 min of steam pretreatment. These results showed that autohydrolysis over several minutes promoted removal of hemicelluloses and degradation or modification of lignin, thereby increasing cellulose content.

Raw EFB1 and EFB2 have relatively similar holocellulose and similar ash content but are dissimilar in lignin content. Raw EFB1 contained the highest lignin compared to all samples. The higher percentages of ash for EFB1 and EFB2 were almost similar to that reported by Simarani et al. [4]. The significantly low percentage of ash content for EFB3 and EFB4 were related to the washing and drying process for preparing these samples. Combined actions like hammering and washing apparently had removed some of the minerals in the EFB as supported by another researcher [15]. In Malaysia, there are two types of EFB generated after the threshing process. There are bunches and shredded EFB as final solid process residues from the mill processing. Since EFB3 and EFB4 are from a readymade shredded EFB within the mill operation, their open structures with high moisture and residual oil contents are easily contaminated by fungus growth which later has detrimental effects on EFB such as deterioration in its properties. Therefore, their biomass handling and transportation to the steam pretreatment equipment need to be clean. Although the chemical compositions of the different types of biomass used were varied in this study and were changed after the pretreatment, a biomass conversion was an accurate measurement or a comparable parameter in

Table 1 – Chemical compositions of raw and steam pretreated EFB.

Sample/Pretreatment time	Chemical composition (g Kg-1 EFB)				
	Cellulose/ (Glucan)	Hemicellulose/ (Xylan + Arabinan)	Lignin	Holocellulose	Ash
EFB1					
Raw/Control[a]	304.7	299.0	206.2	603.7	77.8
15	321.6	294.6	187.2	616.2	64.7
30	333.8	287.5	187.5	621.3	66.8
45	353.0	271.3	179.1	624.3	62.6
60	358.3	274.5	184.0	632.7	63.9
EFB2					
Control[b]	360.2	247.7	174.0	607.9	77.8
15	426.1	208.4	163.7	634.5	53.8
30	403.6	210.0	152.4	613.5	74.0
45	405.8	216.0	150.5	621.8	53.7
60	397.1	220.9	149.9	618.0	53.2
EFB3					
Control[c]	440.1	321.8	173.6	761.9	12.2
15	468.8	293.4	153.8	762.1	11.8
30	458.1	274.2	161.0	745.2	12.3
45	471.1	274.2	154.8	745.2	12.3
60	470.9	279.8	156.2	750.1	13.7
EFB4					
Control[d]	413.4	328.7	189.0	742.0	11.9
15	428.1	284.2	177.2	712.3	13.5
30	421.3	271.9	175.9	693.2	12.1
45	434.0	273.9	176.1	707.8	12.8
60	423.0	270.1	176.2	693.0	13.4

a Raw sample from FFB.
b As received after steam sterilization.
c Sample undergone steam sterilization, shredding, washing and drying.
d Sample undergone steam sterilization, shredding, washing, drying and grinding.

assessing the effectiveness of hydrolysis as stated in section 3.2.

The effects of autohydrolysis on the composition of samples were more pronounced in EFB2 with an 18% increase in cellulose, 16% reduction in hemicellulose and 10% reduction in lignin. This was due to the exploding effect of the EFB structure with high water content present in EFB2 (679 g kg^{-1} EFB2) which rapidly contacted at the high temperature and pressure of the steam which made the biomass structure to be slightly fractionated. The removal of hemicellulose parts is due to the organic acid generation by hemicelluloses acetyl groups' cleavage. The evolved acid was then hydrolyses some of the hemicellulose and alters the lignin structure. The removal of hemicelluloses from the microfibril is believed to expose the cellulose surface and increase enzyme accessibility to the cellulose microfibrils [17].

The EFB3 and EFB4 had the least effect on the chemical compositional changes with this method of pretreatment. A similar increment (7%) in cellulose content with significant ($P < 0.05$) reductions of about 9% and 14% of hemicellulose and lignin respectively, were also obtained in steam pretreatment studies on shredded EFB by Ariffin et al. [2]. This study had a better result as only 15 min instead of 75 min of steaming is used in order to obtain the same effect of the chemical compositional changes of EFB3.

3.2. *Effects of pretreatment time on enzymatic saccharification of different types of EFB*

The enzymatic saccharification is the key step in converting cellulose into ethanol. Studies on the effects of pretreatment time on different types of EFB were conducted to test which pretreatment condition gave the highest sugar production through saccharification. The data presented in Fig. 2 showed the holocellulose conversion rate in different types of EFB with the highest productivity after a 24 h period of enzymatic saccharification. This conversion rate was calculated by dividing the obtained reducing sugar concentration with the potential sugar concentration in the EFB, multiplied by 100. As can be seen from the figure, the highest conversion rate was found in EFB2 followed by EFB3, EFB4 and EFB1 with all raw or control and steam pretreated samples. The conversion rate for EFB2 reached from about 25% to up to 31% and there was a statistically significant difference ($P < 0.05$) between EFB2 for control and pretreated samples as determined by one-way ANOVA. Without additional steam treatment, EFB2 could enhance the holocellulose conversion rate by 25% compared

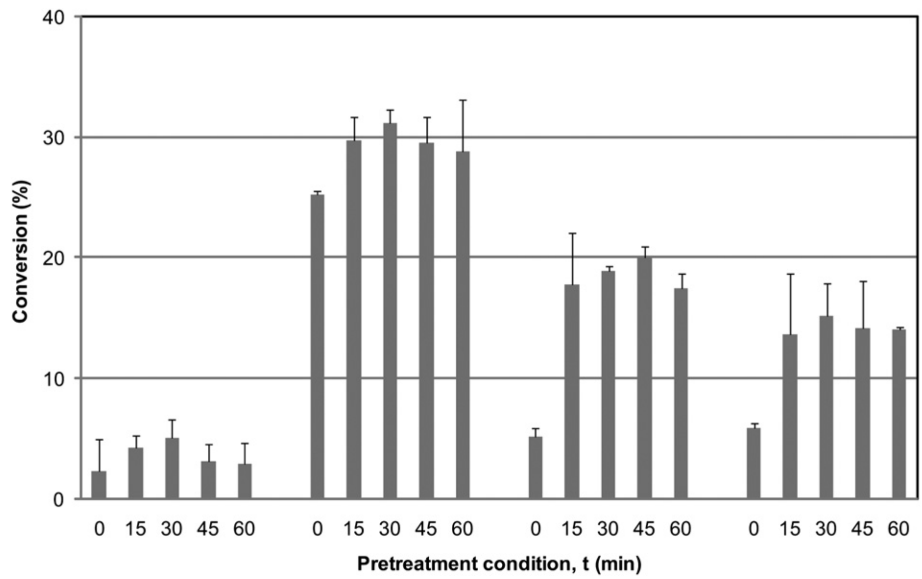

Fig. 2 – Holocellulose conversion of different EFB types and pretreatment times after 24 h of enzymatic saccharification.

to raw EFB1 (2.4%). In the palm oil mill, the EFB2 for control is already steam treated by the ordinary sterilizing process. During sterilization it is important to ensure the evacuation of air from the sterilizer because air could act as a barrier to heat transfer. Therefore, a compact of FFB bunches run in the mill sterilizer would be one of the critical factors to the success of modified structures for easy enhancement of saccharification yield for EFB2 (control). A small quantity of EFB1 with additional steam pretreatment might not respond in the same manner of well contacting between samples for better heat transfer in the mill sterilizer.

The addition of steam to raw and control of EFB1, EFB2, EFB3 and EFB4 had a positive effect and resulted in statistically significant difference ($P < 0.05$) between types of EFB on increased yield of saccharification. When high pressure steam was used for sterilization, the heat caused the moisture in the EFB to expand or explode and hydrolyzed part of the EFB component. Besides that, the moisture introduced by the steam acted chemically to break down gums and resins into soluble and insoluble oils which loosened the fibrous EFB structures for ease of attack by enzymes to be converted to sugars. Besides that, some starches present in the FFB were hydrolyzed and removed by the steam pretreatment therefore increasing the surface area of the biomass for enzymatic attack. By an additional 15 min of steam pretreatment, the holocellulose conversion rate for EFB2 reached up to around 30%. Prolonging the pretreatment time beyond 15 min did not show much improvement and tended to reduce the conversion rate a bit after 60 min of steam pretreatment. The reduced conversion rate is due to changes in the composition of the EFB. A little reduction of the percentage of hemicellulose and lignin in the EFB2 after 60 min of steam pretreatment compared to 15 min of pretreatment gave a good explanation to the decreased saccharification. Hence, it was concluded that 15 min steam pretreatment was the best pretreatment time for most type of EFB.

According to Overend and Chornet [18], the severity of steam pretreatment could be expressed by a single parameter $R0$ as the corresponding values to steam pressure during steam pretreatment which is defined as $R0 = t.exp^{(T-100)/14.75}$, t = the time of reaction (minute), T = temperature of reaction. In their study, the hemicellulose was hydrolyzed even at very low severity. In this study, EFB2 to EFB4 had all undergone significant autohydrolysis in the mill sterilizer by a severity factor of 3.13. Further steaming at different conditions with corresponding values of the severity factor between 2.4 and 3.0 on those samples was able to significantly increase ($P < 0.05$) the conversion rate from around 14%–30%. This increment was related to the modified structure of all the EFB types as these samples might not be organized in the same way anymore after being steam sterilized in an ordinary mill sterilizer. This was demonstrated elsewhere in this study. The severity factor attributed to the deacetylation process of

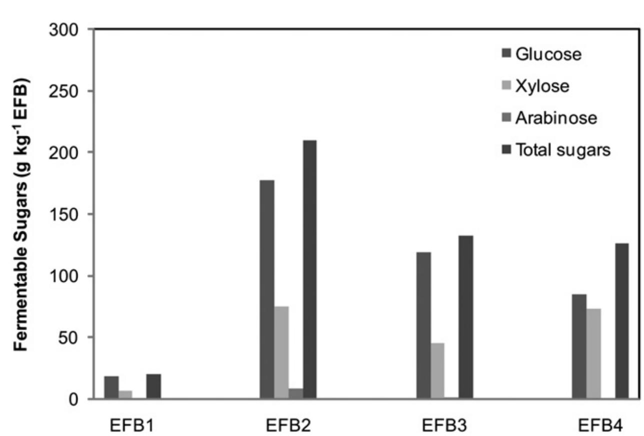

Fig. 3 – Sugars (monosaccharides) released after 24 h of enzymatic saccharification from various samples with 15 min steam pretreatment.

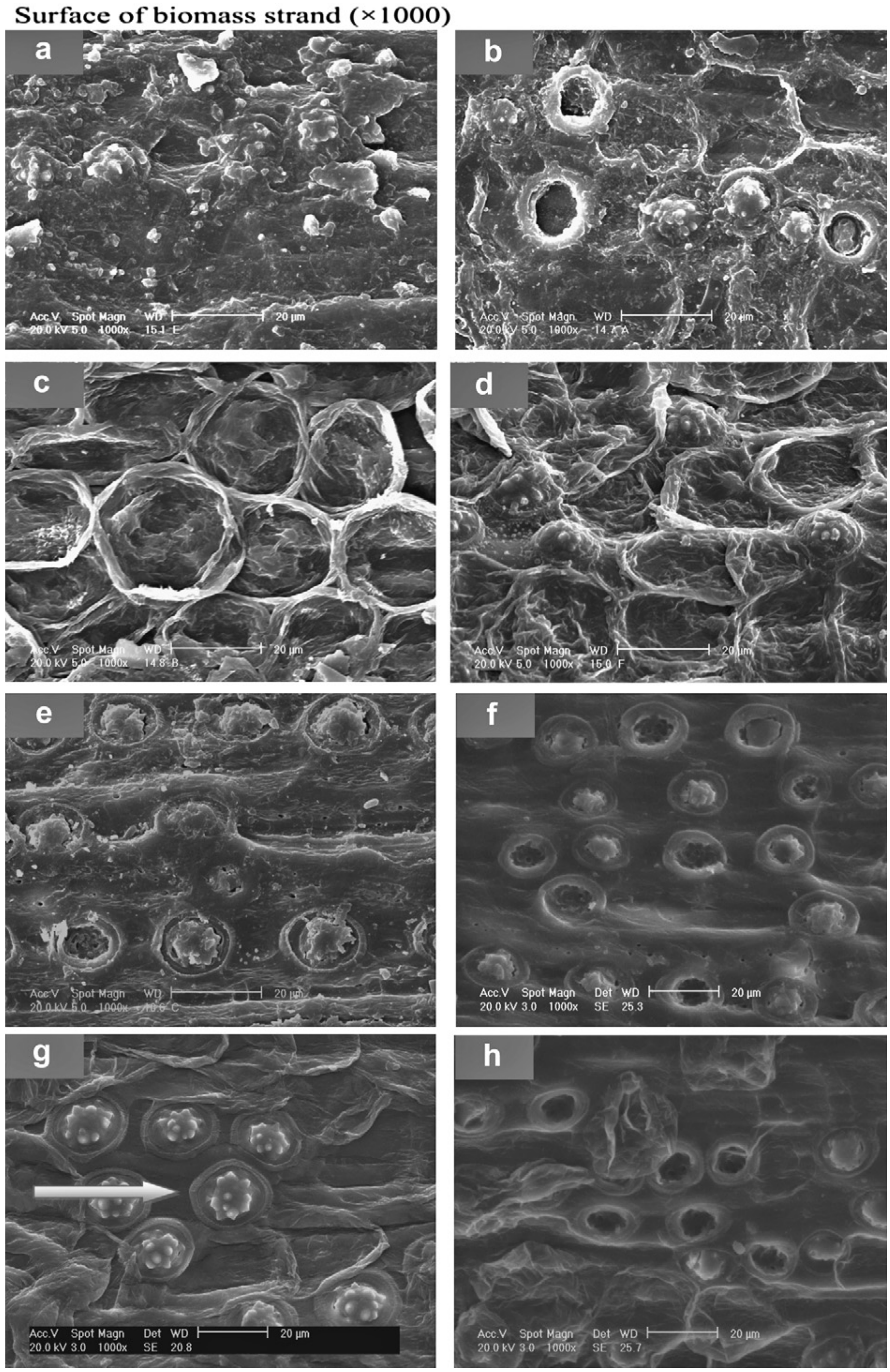

Fig. 4 – Morphological changes of EFB surfaces before and after steam pretreatment. (a, c, e, g) the surface strand of raw sample EFB1-4; (b, d, f, h) the surface strand of pretreated sample EFB1-4; Arrow indicates silica bodies in the EFB strand.

hemicelluloses, autohydrolysis by acids of biomass acetyl groups and the redistribution of lignin by hemicellulose removal might not be the only factor in the increased digestibility of the biomass. There might also be contribution by external factors such as the substrate and enzyme concentrations used with specific enzymatic activities which were believed to increase the biomass conversion effectively after the steam pretreatment process and hydrolysis. For example increasing the severity factor from 2.3 to 2.9 would give 48.8% increase of sugar yields [19].

3.3. *Effects of autohydrolysis on release of sugars from different EFB types*

Overall, the use of steam autohydrolysis as pretreatment increased total sugar production of EFB2, EFB3 and EFB4 by

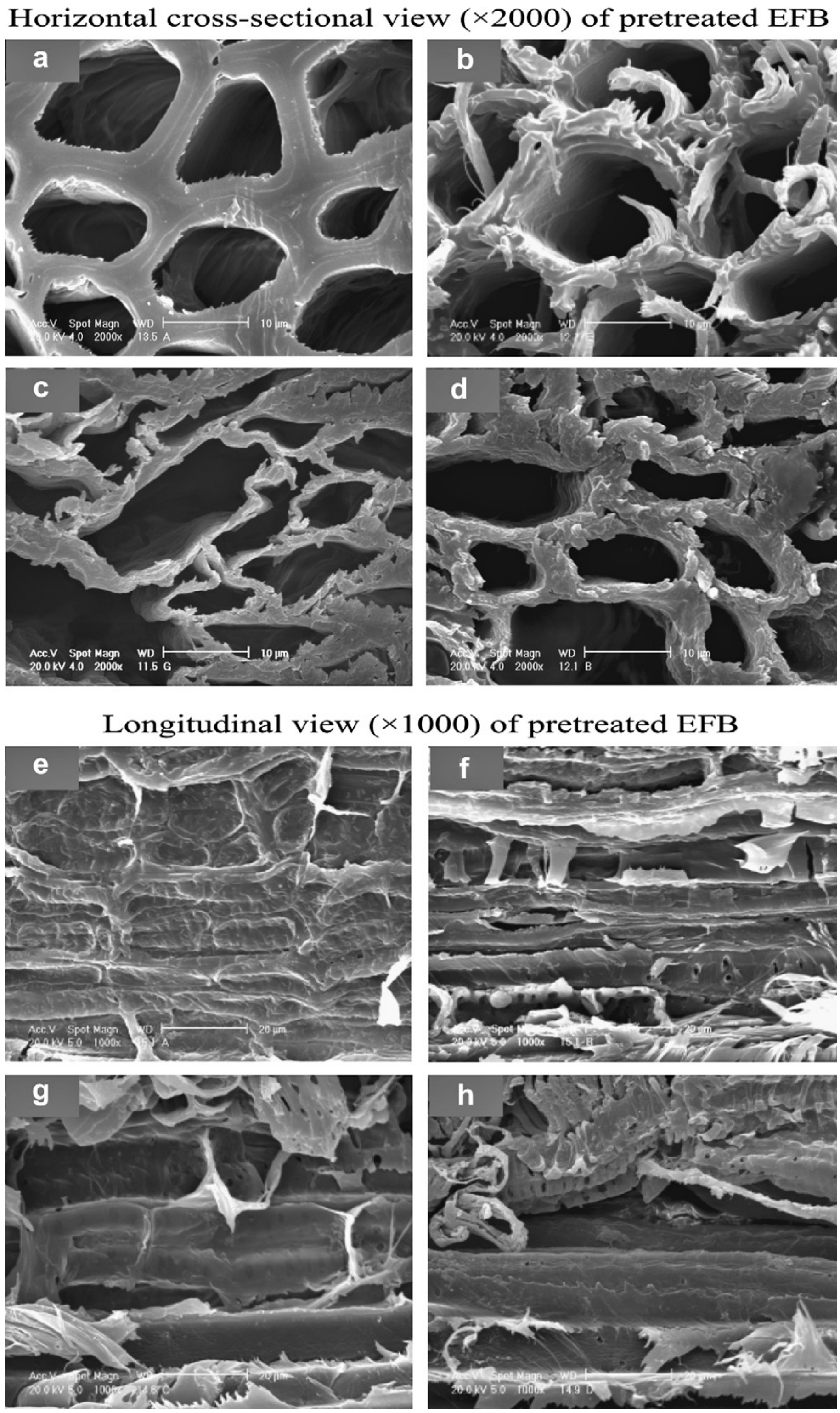

Fig. 5 – Morphological changes of steam pretreated EFB. (a–d) horizontal cross section of middle strand pretreated sample EFB1-4; (e–h) longitudinal section of middle strand pretreated sample EFB1-4.

1.2–3.4 folds over the raw and control samples. The total sugars produced by pretreated EFB1 was 20 g kg^{-1} EFB1. The overall productivity of total sugars and glucose production by EFB1 were 0.83 g kg^{-1} h^{-1} and 0.77 g kg^{-1} h^{-1}, respectively. The saccharification of pretreated EFB2 yielded 209.4 g kg^{-1} EFB2 of sugars with the productivity of total sugars and glucose being 8.7 g kg^{-1} h^{-1} and 7.4 g kg^{-1} h^{-1}, respectively.

The sugars produced from pretreated EFB3 and EFB4 were 132.2 g kg^{-1} EFB3 and 0.126.3 g kg^{-1} EFB4, respectively. The yield of pretreated EFB4 was slightly improved by 8% when compared to the finding obtained by Ariffin et al. [2] with the same steam pressure. The productivity of total sugars and glucose were 5.5 g kg^{-1} h^{-1} and 4.9 g kg^{-1} h^{-1} for EFB3 and 5.3 g $kg^{-1}h^{-1}$ and 3.5 g kg^{-1} h^{-1} for EFB4, respectively. Fig. 3 shows the results of sugar yields after saccharification of EFB1, EFB2, EFB3 and EFB4.

3.4. *Effects of morphological alterations in EFB by steam autohydrolysis pretreatment*

Fig. 4 shows micrographs of raw and steam pretreated EFB surfaces examined under the scanning electron microscope. From this figure, obvious differences could be observed on EFB surfaces after 15 min sterilization of the raw samples. The raw fresh EFB (EFB1) in Fig. 4a initially had a rough surface with a layer of matrix material like lignin or waxes that covered the whole surface strand. This layer might be the cuticle or the protective layer present in most plants to prevent water loss. This is the only micrograph of EFB that had not yet been in contact with steam. Therefore, the structure looked very rigid and solid. The EFB1 from the same bunch that had undergone steaming for 15 min showed a marked change (Fig. 4b). Some of the materials on the structure seemed to be removed by the pressure and heat from the steam. As a result, the surface looked smooth, clear and showed some of the silica bodies located behind the first layer.

Noticeable difference could be found in the control sterilized EFB (EFB2) micrograph (Fig. 4c). In this picture, another thin porous layer could be seen attached together; round in shape over the EFB strand surfaces resembling a polyurethane structure in a micrograph captured by Shamsudin et al. [20]. This coated matrix resulted after the 90 min sterilization process in palm oil production. This layer seemed to vanish after being compressed by steam and part of its concealed silica bodies became exposed (Fig. 4d).

The surface characteristics of raw EFB3 and EFB4 are shown in Fig. 4e and g, respectively. Both types had quite similar patterns of features wherein silica bodies were found scattered all over their surfaces after being washed. The average diameter of these round-shaped silica bodies was found to be 15 μm, in line with the observations by Law et al. [15]. Steam pretreatment on EFB3 and EFB4 resulted in dislodging of the silica bodies and revealed craters with perforated bottoms on the strand surfaces (Fig. 4f and h). Even though the silica bodies in the EFB could be the limitation to the cellulose conversion, their removal did not significantly increase the hydrolysis conversion, as only up to 17.8% conversion was obtained by the steam effect.

Fig. 5 shows the effects on EFB morphology from horizontal cross sectional and longitudinal views of pretreated samples. One could see that the strands of EFB were the aggregates of rod-shaped microfibrils. The horizontal cross sectional micrograph Fig. 5(a–d) showed that there were cavities in the middle of the fiber and this was supported by others [15,16]. The porous cell walls were found to shrink and became softer after the steaming process. The highest total conversion by steam pretreated EFB2 which was higher than EFB3 and EFB4 could be due to the existence of pores in the EFB2 seen in the cross sectional view micrograph (Fig. 5b), which facilitated the penetration of enzyme and enhanced the digestibility of the biomass therefore improving the enzymatic hydrolysis. Since the pore volume of fiber was directly correlated with accessibility to hydrolytic enzyme, therefore the shrunken pores in EFB3 and EFB4 (Fig. 2c and d) could explain the reduced conversion. This phenomenon could be described as hornification; led by water loss due to pore shrinkage in fiber [9]. The drying process during the preparation of EFB3 and EFB4 reduced the number of larger pores in the fiber and subsequently, the conversion of biomass into sugars. A rigid cell wall of pitted vessel elements was observed from the longitudinal view of EFB1 (Fig. 5e) and it changed to a destroyed cell wall of pitted vessels (Fig. 5f–h) after the sterilization process.

3.5. *Mass balance calculation for sugars production*

The mass balance for steam pretreated EFB2 under pressure is illustrated in Fig. 6. Mass balance calculation is important to determine the energy used, produced and the excesses in the system employed for EFB as substrate for sugars and subsequent ethanol production. Steam pretreatment under 140 °C/ 0.28 MPa resulted in parts of the holocellulose in terms of cellulose with hemicellulose, lignin and miscellaneous fractions being solubilised to the condensate. Two hundred and fifty gram samples of EFB2 contained 80.3 g dry weight of EFB and 48.8 g carbohydrate. After 15 min of steam pretreatment, 15% of dry weight of the sample was lost due to many circumstances. Direct steam exposure had removed some of the minerals in the biomass which reduced the percentage of residues left after combustion (ash). This reduction contributed to 2.4% loss. Another 3.5% reduction resulted from lignin

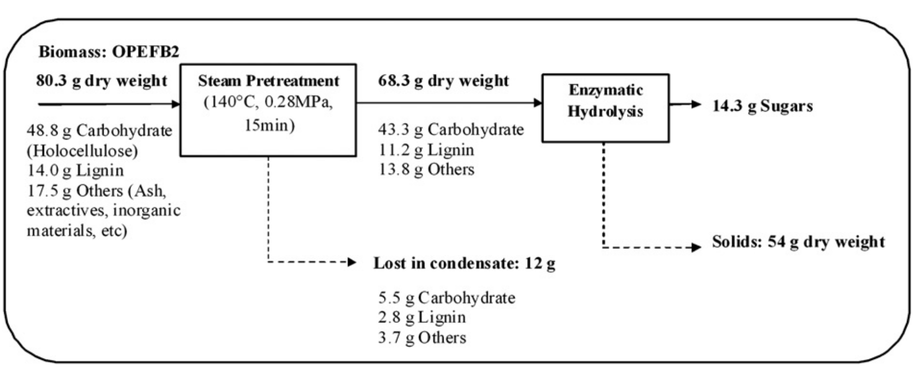

Fig. 6 – Mass balance results for EFB2 after 15 min steam pretreatment.

removal and 6.8% from the solubilised carbohydrate component. After enzymatic saccharification, about 18% (14.3 g) sugar was produced through this system as sugar recovery.

In the palm oil mill, the boilers generate saturated and superheated steam from 140 °C to 260 °C. There is about 55.5 Gg excess saturated steam (140 °C) produced in the mill which is equivalents to 12.6% of energy loss as steam from the sterilizer exhaust. Pretreatment by the ordinary sterilizing process in the mill has been proven to increase glucose production by 44.6% [4]. From this study, introduced additional steam to EFB2 with moisture content of 679 g kg^{-1} EFB2, had increased sugar recovery to 23.3% with glucose as the main sugar. Hence, the mill's saturated steam had a positive impact by increasing yield after saccharification. Thus, future investigation should be done to investigate the effects of various temperatures, durations and moisture contents to further optimize the conversion process by saturated steam.

4. Conclusion

In this study, the autohydrolysis process as a pretreatment which involved steam and water was shown to upgrade the enzymatic digestibility of EFB2 by the partial removal of hemicellulose. The saccharification percentage by fresh sterilized EFB (EFB2) was increased up to 1.8 folds compared to shredded (EFB3) and ground EFB (EFB4). Overall, the steam pretreatment (0.28 MPa/140 °C) increased sugar production from 1.2 to 3.4 fold compared to raw EFB. Steam pretreatment in the context of palm oil milling is an attractive approach because it does not require the addition of chemicals and it can be operated directly after the fruit has been stripped off for oil production. In this way, the EFB can be immediately processed and saccharified into sugars for subsequent ethanol production as part of the mill operation.

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REFERENCES

[1] Wahid MB. Overview of the malaysian oil palm industry 2009 [report on the Internet]. Selangor, Malaysia: Malaysian Palm Oil Board. Available from: http://econ.mpob.gov.my/economy/Overview_2009.pdf; 2010 Jan [cited 2011 Aug 28].

[2] Ariffin H, Hassan MA, Umi Kalsom MS, Abdullah A, Shirai Y. Effect of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB). J Trop Agric Fd Sc 2008;36(2):259–68.

[3] Seong KS, Hassan MA, Ariff AB. Enzymatic saccharification of pretreated solid palm oil mill effluent and oil palm fruit. Pertanika J Sci Technol 2008;16(2):157–69.

[4] Simarani K, Hassan MA, Abd-Aziz S, Wakisaka M, Shirai Y. Effect of palm oil mill sterilization process on the physicochemical characteristics and enzymatic hydrolysis of empty fruit bunch. Asian J Biotechnol 2009;1(2):57–66.

[5] Ramos LP. The chemistry involved in the steam treatment of lignocellulosic materials. Quim Nova 2003;26(6):863–71.

[6] Kaar WE, Gutierrez CV, Kinoshita CM. Steam explosion of sugarcane bagasse as a pretreatment for conversion to ethanol. Biomass Bioenerg 1998;14(3):277–87.

[7] Linde M, Jakobsson EL, Galbe M, Zacchi G. Steam pretreatment of dilute H_2SO_4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production. Biomass Bioenerg 2007;32:326–32.

[8] Öhgren K, Bura R, Saddler J, Zacchi G. Effect of hemicelluloses and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. Bioresour Technol 2007;98: 2503–10.

[9] Sun F, Chen H. Enhanced enzymatic hydrolysis of wheat straw by aqueous glycerol pretreatment. Bioresour Technol 2008;99:6156–61.

[10] Mohamed H. Application of theoretical combustion analysis in determining the optimum fibre/shell ratio for oil mill boiler [report on the Internet]. Selangor, Malaysia: Universiti Putra Malaysia. Available from: http://psasir.upm.edu.my/120/; 2006 Jan [cited 2011 Sept 5].

[11] Alvira P, Tomás-Pejó E, Ballosteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 2010;101:4851–61.

[12] Baharuddin AS, Wakisaka M, Shirai Y, Abd-Aziz S, Abdul Rahman NA, Hassan MA. Co-composting of empty fruit bunches and partially treated palm oil mill effluents in pilot scale. Int J Agric Res 2009;4(2):69–78.

[13] AOAC. Method 973.18, fiber (acid detergent) and lignin in animal feed. Arlington. In: Official methods of analysis of AOAC international. 16th ed.; 1997. p. 28–9.

[14] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. J Anal Chem 1959;31:426–8.

[15] Law KN, Wan Daud WR, Ghazali A. Morphological and chemical nature of fiber strands of oil palm empty fruit bunch (OPEFB). Bioresources 2007;2(3):351–62.

[16] Sreekala MS, Kumaran MG, Thomas S. Oil palm fiber: morphology, chemical composition, surface modification and mechanical properties. J Appl Polym Sci 1997;66:821–35.

[17] Gupta RB, Demirbas A. Gasoline, diesel and ethanol biofuel from grasses and plants. In: Ethanol from biomass by fermentation. United States of America: Cambrige University Press; 2010. p. 84–101.

[18] Overend RP, Chornet E. Fractionation of lignocellulosics by steam-aqueous pretreatments. Philos T Roy Soc A 1987;321: 523–36.

[19] Lamptey J, Robinson CW, Moo-Young M. Enhanced enzymatic hydrolysis of lignocellulosic biomass pretreated by low-pressure steam autohydrolysis. Biotechnol Lett 1985; 7(7):531–4.

[20] Shamsudin S, Kalil MS, Wan Yusuf WM. Production of acetone, butanol and ethanol by *Clostridium saccharoperbutylacetonicum* N1-4 with different immobilization systems. Pakistan J Biol Sci 2006;9(10): 9123–8.