Combination of Pre-treatments on EFB Fibre for Sugar Production

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INTRODUCTION

Oil palm empty fruit bunches (EFB) contain about 70%-80% holocellulose, which comprises about 40%-45% and 30%-35% of cellulose and hemicellulose, respectively, as well as 18%-22% lignin (Basiron and Husin, 1996). These lignocellulosics can be utilised for the production of fibre-reinforced biocomposites (Mariko *et al.*, 2016) and fine chemicals such as glucose and xylose (Siew *et al.*, 2013). Due to their high content of cellulose and hemicellulose, EFB can be exploited for the extraction of high value-added lignocellulosic fine chemicals. One of the strategies is to convert the cellulose and hemicellulose into fermentable sugars such as glucose and xylose (Astimar *et al.*, 2000a, b).

However, the direct conversion of these lignocellulosics into simple sugars is challenging. The three main factors that affect the conversion process are the content of lignin and hemicellulose, the crystalline structure of the cellulose, and the accessibility of the surface area (Hongzhang, 2014). Hence, a pre-treatment process is important to enhance the conversion of lignocellulosic materials into simple sugars.

The basic pre-treatment processes available are classified as physical, chemical and biological. Physical treatment such as grinding, milling, chipping and shredding which increases surface area or reduces the degree of polymerisation and cellulose crystallinity, and chemical treatment with acid or alkali, are commonly used. Acid pre-treatment has a solubilising effect on hemicellulose while alkaline pre-treatment has been known to be effective in the delignification process (Marcotullio *et al.*, 2011). However, a combination of both treatments could bring about more efficiency to the pre-treatment process and enzymatic hydrolysis. Furthermore, enzymatic hydrolysis is influenced by several factors such as lignin and hemicellulose contents, cellulose crystallinity, degree of polymerisation, accessible surface area and pore volume. However, lignin forms present physical barriers that restrict access of the enzyme to the cellulose. Selective removal of lignin during delignification would minimise cellulose degradation and thus enhance the enzymatic saccharification processes (Chang and Holtzapple, 2000; Zhao *et al.*, 2007; Seung *et al.*, 2015).

Plenty of combined treatments have been applied to pre-treat various lignocellulosic materials, and these include steam explosion or hydrothermal treatment combined with alkali pre-treatment (Chen *et al.*, 2008; Cuevas *et al.*, 2014; Sun *et al.*, 2014a), mild physical or chemical pre-treatment combined with biological pre-treatment (Yu *et al.*, 2009), and dilute acid combined with alkali pre-treatment (Lee *et al.*, 2015).

Cuevas *et al.* (2014) discovered that 60% of lignin was removed during the subsequent alkaline peroxide process, and that the digestibility of the residues after the combined pre-treatment was significantly improved as compared with the residues after hydrothermal pre-treatment alone. Meanwhile, Sun *et al.* (2014b) also investigated the feasibility of steam explosion combined with alkali pre-treatment. It was found that steam explosion combined with alkali pre-treatment enhanced the rate of enzymatic hydrolysis by 45.7%-63.9%, while steam explosion alone improved the enzymatic hydrolysis rate by 7.9%-33.1% only. Thus, an effective treatment should be able to extract these chemicals separately while maintaining their natural characteristics, and use less chemicals, thus producing less environmentally hazardous wastewater.

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The objective of this work is to investigate the fractionation of EFB fibre by means of physico-chemical processes, in enhancing its susceptibility towards enzymatic hydrolysis for the production of fermentable sugars (glucose and xylose).

MATERIALS AND METHODS

Samples of EFB fibre were collected from the Palm Oil Milling Technology Centre (POMTEC), which is located in Labu, Negeri Sembilan, Malaysia. Green EFB fibre were then transported to the MPOB/UKM Research Station in Bangi Lama, Selangor, Malaysia for further processing.

Pre-treatment of EFB

At the research station, the EFB fibre were dried in an electric oven at 105°C ± 2°C until 10%-15% moisture content. About 10 kg of dry EFB fibre were soaked in NaOH solution (varying from 5%-30% in concentration) at a ratio 1:10 (w/v), and left overnight at ambient temperature. After soaking, the EFB fibre were defibrated in a single-disk pressurised refiner (Thermo mechanical refiner, Andritz 12") at 3000 rpm under 2 bar pressure. The cooking conditions used were as follows: retention times of 200, 400 and 600 s, and temperatures of 150°C, 160°C and 170°C. All the steps of the experiment are shown in the flow chart in *Figure 1*.

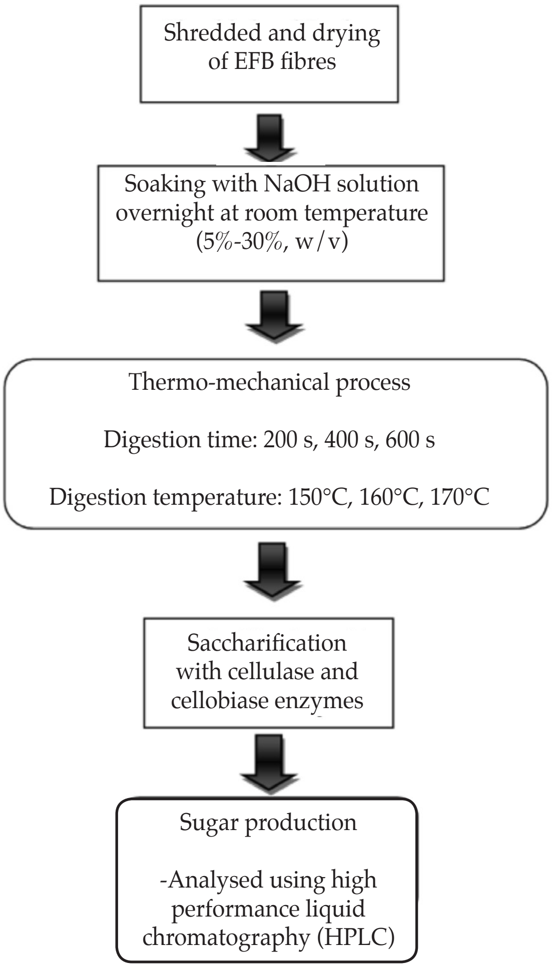

Figure 1. The flow chart of the experiments.

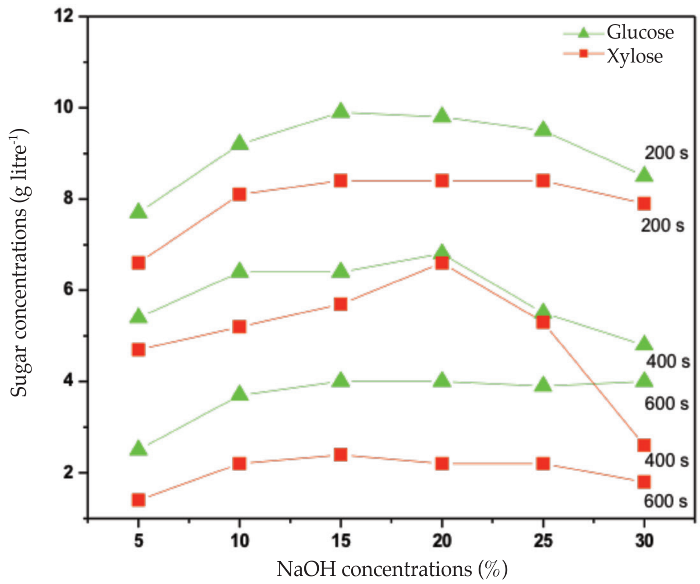

Figure 2. Effect of retention time of (a) 200 s, (b) 400 s and (c) 600 s on the extraction of fermentable sugars with various sodium hydroxide (NaOH) concentrations (digestion temperature of 150°C).

RESULTS AND DISCUSSION

Effects of Retention Time

The effect of retention time on the extraction of fermentable sugars, at the various concentrations of sodium hydroxide (NaOH), is shown in *Figure 2*. It was observed that sugar yield increased with an increase in NaOH concentration. The highest yield of sugar obtained was 23.6 g litre^{-1} (comprising 15.2 g litre^{-1} of glucose and 8.4 g litre^{-1} of xylose) from EFB fibre treated with 20% NaOH for 200 s of digestion time, while, after treatment with 20% NaOH, the sugar yield decreased with increasing digestion time. According to Ye and Jiayang (2002), this is due to some portions of the sugars being further transformed into furans (furfural and hydroxyl-methyl furfural) and other by-products, which leads

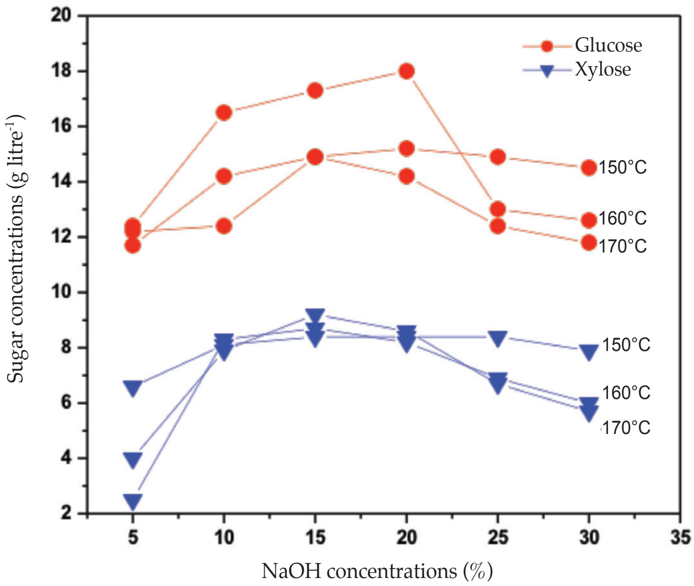

Figure 3. Effect of digestion temperatures of (a)150°C, (b) 160°C and (c) 170°C on the extraction of fermentable sugars with various sodium hydroxide (NaOH) concentrations (digestion period of 200 s).

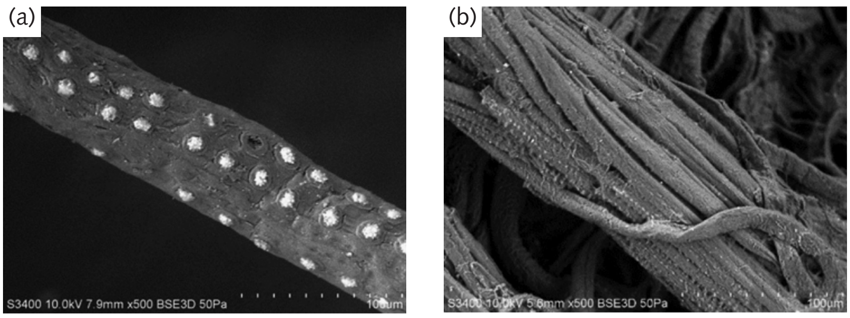

Figure 4. Scanning electron microscopy (SEM) micrographs (500X) of (a) untreated empty fruit bunches (EFB) and (b) EFB treated with 20% NaOH for 200 s (at digestion temperature of 160°C).

to the loss of sugars after alkaline treatment. The pre-treatment process is needed to remove the lignin which can reduce crystallinity and degree of polymerisation of the cellulose, while increasing the surface area for accessibility to enzyme activity (Ayyachamy *et al.*, 2013; Zhong *et al.*, 2014). The current finding is in agreement with that of Ishiguro and Endo (2014) who used hydrothermal-mechano-chemical pretreatment. Based on the results, the optimum parameters for this pre-treatment process are subjecting the fibre to 20% NaOH for 200 s.

Effects of Digestion Temperature

Figure 3 shows the effect of digestion temperature (150°C, 160°C and 170°C) on sugar yield. The highest sugar yield was 26.2 g litre^{-1} (18.0 g litre^{-1} glucose and 8.2 xylose g litre^{-1}) which occurred at 160°C with 20% NaOH concentration. The amount of sugar increased along with increasing NaOH concentration until 20% concentration, after which the yield started to decrease. It is understood that an effective thermal treatment will eliminate hemicellulose, enhance the delignification process and increase the crystallinity of cellulose (Mood *et al.*, 2013; Harun *et al.*, 2016). This process also increases the total surface area of the treated fibre and their accessibility to enzymatic hydrolysis in sugar production (Pauline, 2008).

Microstructure Analysis

Figure 4 shows the microstructure of the untreated EFB fibre and the fibre treated with 20% NaOH solution. The NaOH treatment showed significant effects on the microstructure of the EFB fibre. The formation of strands was observed, indicating that the structure had been loosened up due to the delignification process, in that the bundles of EFB microfibrils were swollen by NaOH. As lignin acts like a glue in the plant cell wall (Tina, 1998), when delignification occurred, the bonding between cellulose strands was loosened up. Therefore, this enhanced the accessibility of the components to the enzymatic saccharification to produce fermentable sugars (Brodeur *et al.*, 2011). The original surface of the untreated EFB fibre was also seen to have some particles attached in the pores. After the NaOH treatments, the particles seemed to have leached away. It has been reported in another study that those particles are from the minerals and extractives of the fibre (Jiang *et al.*, 2013), while the removal of hemicellulose resulted in increases in pore volume and accessible surface area (Palonen, 2004).

It was also observed that the fibre treated with NaOH concentrations higher than 20% became severely disintegrated, which might have resulted in damage of the cellulose and hemicellulose, thus reducing the availability of both components for enzymatic saccharification to produce fermentable sugars as shown in the results earlier (*Figures 2* and *3*). Thus, the combination pre-treatment was able to successfully change the morphology of EFB fibre, thus affecting the accessibility of the enzyme into the lignocellulosic biomass for fermentable sugar production.

CONCLUSION

The combined pre-treatment, which is a physico-chemical process, successfully influenced the morphology of EFB fibre, thus enhancing the enzymatic activity on the EFB fibre towards sugar production. The optimised physico-chemical reaction parameters for the EFB fibre were 20% concentration of NaOH, 160°C digestion temperature and 200 s digestion period. The highest yield of sugar was 26.29 g $litre^{-1}$ (18.01 g $litre^{-1}$ of glucose and 8.28 g $litre^{-1}$ of xylose). It can be implied from this study that the combined pre-treatment process can be used for the production of sugars from EFB, and that this system can be integrated into the medium density fibreboard (MDF) plant. Production of fine chemicals from the extracted sugars will benefit the MDF industry, thus making it economically viable.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article.

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