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Combined pretreatment using alkaline hydrothermal and ball milling to enhance enzymatic hydrolysis of oil palm mesocarp fiber

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HIGHLIGHTS

- Oil palm mesocarp fiber suitable lignocellulosic biomass for biosugar production.
- Hydrothermal treatment improved hemicellulose removal and lignin migration.
- Alkaline hydrothermal treatment improved ester bond cleavage and delignification.
- Mechanochemical treatment reduced particle size and crystallinity of cellulose.
- The highest xylose and glucose obtained were 63.2% and 97.3%.

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ABSTRACT

Hydrothermal pretreatment of oil palm mesocarp fiber was conducted in tube reactor at treatment severity ranges of log *Ro* = 3.66–4.83 and partial removal of hemicellulose with migration of lignin was obtained. Concerning maximal recovery of glucose and xylose, 1.5% NaOH was impregnated in the system and subsequent ball milling treatment was employed to improve the conversion yield. The effects of combined hydrothermal and ball milling pretreatments were evaluated by chemical composition changes by using FT-IR, WAXD and morphological alterations by SEM. The successful of pretreatments were assessed by the degree of enzymatic digestibility of treated samples. The highest xylose and glucose yields obtained were 63.2% and 97.3% respectively at cellulase loadings of 10 FPU/g-substrate which is the highest conversion from OPMF ever reported.

1. Introduction

Bioethanol derived from biomass has been recognized as a potential substitute to fossil fuel since biomass is abundant in nature, non-food competitive and sustainable. Oil palm industry has contributed the highest percentage of biomass generated from oil palm processing. It was estimated approximately 96.0 Mt of fresh fruit bunches (FFB) has been processed and amounted 19.2 Mt crude palm oil (CPO) production (Malaysian Palm Oil Board, 2014). Oil palm mesocarp fiber (OPMF) is one of lignocellulosic biomass generated from oil palm processing and consist mixtures of exocarp (outer skin), mesocarp (pulp) and crushed endocarp (shell). One ton of FFB could generates 0.12 ton of OPMF and by this calculation, it is projected about of 11.5 Mt was generated in 2013 (Malaysian Palm Oil Board, 2014). In normal practice this materials were used as a source of fuel in a boiler system to produce energy for the mill's internal use. Under Clean Development Mechanism (CDM), OPMF has been used as a source of carbon in composting process and in anaerobic digester to improve methane production. OPMF are lignocellulosic complex composites of cellulose, hemicellulose and lignin like other plant biomass. Natural recalcitrance of lignocellulosic biomass complex hinders the accessibility of enzyme in hydrolysis process thus limit the production of pentose and hexose sugars. Four steps are involves in biochemical routes of bioconversion of biomass to ethanol production; (1) pretreatment of lignocellulosic biomass, (2) enzymatic hydrolysis, (3) fermentation and (4) separation of bioethanol and purification (Mosier and Wyman, 2005; Nitsos et al., 2013). The successful of pretreatment step is crucial in determining the fate of future processes.

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Different pretreatment methods have been adopted with the aim to reduce and remove the natural recalcitrance of lignocellulosic biomass to get maximum access of cellulase to cellulose. The available methods may vary from physical, chemical, and thermochemical depending on the types and nature of biomass (Mosier and Wyman, 2005). Comminution process such as planetary/attrition ball milling and wet disk milling has resulted in reduction of particle size, increased surface area, pore volume and reduced crystallinity index (CrI) of cellulose, thus enhancing the enzymatic digestibility of biomass (Hideno et al., 2009; Silva et al., 2010; Liao et al., 2011). Concerning maximal accessibility of cellulase to cellulose, a large portion of hemicellulose and lignin have to be removed from the cellulose–hemicellulose–lignin matrix (Mosier and Wyman, 2005; Mussatto et al., 2008). Hemicellulose can be partially removed through hydrothermal pretreatment such as steam and aqueous (autohydrolysis) methods since these treatments use only water as natural reactant and catalyst under a wide range of temperature and residence time (Möller et al., 2011; Nitsos et al., 2013). The progress of fractionation of lignocellulosic materials are heavily dependent on the intensity of pretreatment, normally expressed as severity factor, log *Ro* (Overend and Chornet, 1989). Precise control of treatment severities may avoid the production of fermentative inhibitors such as acetic acid, furfural and 5-hydroxy-methyl-furfural (HMF) (Overend and Chornet, 1989; Möller et al., 2011; Nitsos et al., 2013). Hydrothermal pretreatment is always associated with the release/migration and re-condensation of lignin in the form of spherical droplets on the surface of pretreated biomass (Donohoe et al., 2008). Formation of pseudolignin from hemicellulose degradation increased the amount of lignin droplets, resulting in 'traffic jam' effect and non-specific binding of lignin on cellulose, which may have a detrimental effect of enzymatic hydrolysis, thus reducing the yield of glucose in cellulose conversion (Donohoe et al., 2008; Selig et al., 2007; Pu et al., 2013).

Recently, combined hydrothermal and subsequent NaOH treatment was conducted to dissolve hemicellulose components and remove the lignin from the pretreated biomass, thus enhancing the enzymatic digestibility of pretreated biomass (Mussatto et al., 2008; Gao et al., 2013; Ishiguro and Endo, 2014). In the present study, a combination of pretreatment methods was performed with the aim to disrupt the organized polymeric structure of OPMF. The treatment efficiency was demonstrated by the high xylose and glucose yields from enzymatic hydrolysis of treated OPMF.

2. Methods

2.1. Preparation of raw materials and componential analysis

Oil palm mesocarp fiber (OPMF) was collected from Serting Hilir Palm Oil Mill, Jempol, Negeri Sembilan, Malaysia. The collected OPMF consisted of mixtures of exocarp (outer skin), mesocarp (pulp), crushed kernel and endocarp (shell) (Fig. S1). The crushed kernels and shells were manually separated from OPMF fibers prior to componential analysis and other experimental work in order to avoid error in the data analysis. Unless otherwise stated, the sample used in this study was in its original size as collected from the mill (20–30 mm). The compositions of extractives, cellulose, hemicellulose, acid soluble lignin, acid insoluble lignin and ash content were determined by a method recommended by Teramoto et al. (2008).

2.2. Mechanochemical activation-ball milling pretreatment

Ball milling (BM) pretreatment was performed according to a method reported by Inoue et al. (2008). Untreated and hydrothermally treated OPMF were treated using the planetary ball mill Pulverisette 5 (Fritsch, Germany). The sample (20 g), was milled at 250 rpm in a 500 mL milling cup with 25 spheres (ψ = 20 mm). Planetary ball mill Pulverisette 7 (Fritsch, Germany) was used for lower amount of treated OPMF. The sample (0.5 g), was milled at 250 rpm in a 45 mL milling cup with 6 spheres (ψ = 5 mm). Milling was carried out for a total time of 60–240 min (with a cycle of 10 min run and 10 min pause) at room temperature. The experiments were performed in duplicate. The BM time indicated in this study refers to the actual milling time, excluding the paused time. Samples were kept *in vacuo* at room temperature prior to enzymatic hydrolysis.

2.3. Alkaline pretreatment

Four gram (4 g) of oven dried OPMF samples were placed in a laboratory bottle with stopper (NEG, Japan) and then mixed with 100 mL of NaOH solution (1.0%, 1.5% and 2.5%). The mixture was incubated at 50 °C for 3 h with stirring. After pretreatment, the solid residue was separated by filtering (Filter paper No. 2, Advantec, Japan) and wash with distilled water until neutral pH. The solid residue was oven dried at 90 °C for 24 h prior to chemical analysis and enzymatic saccharification.

2.4. Hydrothermal pretreatment

Hydrothermal pretreatment of OPMF was conducted in a stainless steel tube reactor (outside diameter, 25 mm; wall thickness, 2 mm; and length, 100 mm). In general, 3 g of oven dried OPMF and 30 ml of distilled water was used to fill the reactor. Solid to liquid ratio (S:L) of 1:10 was used in this study. Fully tightened reactor filled with biomass sample and water was then carefully emerged into a sand bath, which was maintained at temperature range from 180 to 220 °C governed by automatic temperature controller. The reactor was agitated at 60 rpm in order to provide homogenize mixing of samples in the tube reactor. After completion at 20 min residence time the reactor was transferred from sand bath into water reservoir and cooled down to 30 °C. The slurry was withdrawn from the reactor and transferred into 65 mL glass bottles with cap (NEG, Japan) and stored prior to enzymatic hydrolysis. The pH value of the hydrothermally treated OPMF samples was measured using a digital pH meter (D-53, Horiba, Japan).

Alkaline hydrothermal pretreatment was performed by addition of diluted NaOH solution (0.5–2.5%) into a tube reactor at the same S:L ratio as mentioned above. The slurry was filtered using filter paper No. 2 (Advantec, Japan) and washed with distilled water until neutral pH. The neutralized solid was oven dried at 90 °C for 24 h prior to enzymatic hydrolysis. The intensity of the hydrothermal treatment was expressed as severity factor (log *Ro*). The severity parameters corresponding to different hydrothermal pretreatment conditions are calculated as in Eq. (1).

$$Ro = t\exp[(T-100)/14.75] \quad (1)$$

In which t is the reaction time (min), and T is the hydrolysis temperature (°C) (Overend and Chornet, 1989).

2.5. Sieving procedure

Ball milled-treated OPMF samples was separated by using an Analysette 3 vibratory sieve shaker (Fritsch, Germany) with three different sieves size were selected; 2 mm, 500 μm and 250 μm and operated for 10 min at an amplitude of 0.5.

2.6. Fourier transform infrared (FT-IR)

Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer over a range of