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Comparison of steam-alkali-chemical and microwave-alkali pretreatment for enhancing the enzymatic saccharification of oil palm trunk

Long Wee Lai [a], Ani Idris [b, *]

[a] Faculty of Science and Biotechnology, Universiti Selangor, Bestari Jaya 45600, Malaysia
[b] Department of Bioprocess Engineering, Faculty of Chemical Engineering, c/o Institute of Bioproduct Development, Universiti Teknologi Malaysia, UTM, Skudai 81310, Malaysia

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ABSTRACT

This paper demonstrates two different pretreatment protocols for oil palm trunks (OPT); steam-alkali-chemical (SAC) and microwave-alkali (Mw-A) method. The composition, morphology, structure and crystallinity of OPT before and after pretreatment were analyzed. The effectiveness of the pretreated methods was investigated by performing enzymatic saccharification on the OPT. The physiochemical factors namely: enzyme ratio (cellulase to β-glucosidase), pH, temperature and substrate loading (w/v) on enzymatic saccharification were also investigated. The pre-determined optimal conditions were then used for further enzymatic hydrolysis of raw and pretreated OPT substrates. The results revealed a huge degree of reduction in lignin, up to 89% for SAC treated OPT and at least 15% for Mw-A treated OPT sample as compared to untreated ones. High glucose accumulation (79.4%) was obtained after 72 h saccharification for both pretreated OPT samples.

1. Introduction

Lignocellulosic biomaterial is abundantly available throughout the world. It is a low cost renewable feedstock and can be converted into fermentable sugars and served as an intermediate to industrially important compounds [1]. In 2014, Malaysia has about 5.39 million hectares of oil palm (*Elaeis guineesis*) cultivation. An approximate 18 million ton of oil palm trunks (OPTs) are produced annually as a result of the replanting process after 25–30 years [2]. The conversion of OPT biomass into high value-added products can be viewed as a productive disposal. The native lignocellulosic OPT biomass contains high amounts of polymeric carbohydrates which comprise mainly of cellulose, hemicellulose and lignin. The cellulose compounds can degrade into fermentative saccharides (mainly glucose) by either chemical reaction (e.g. acid hydrolysis) or enzymatic saccharification. The formed glucose can also be further transformed into bioethanol and other value-added products [3]. Unlike acid hydrolysis, enzymatic reaction has a number of advantages which includes high product yield, mild reaction conditions, low energy requirement and less pollution [4].

However, the presence of natural recalcitrant components makes enzymatic saccharification towards lignocellulosic substrate more challenging. In order to obtain glucose from the encrusted lingo-biomass, two processes are necessary and inevitable; i) pretreatment and ii) enzymatic hydrolysis. In the pretreatment process the complex cellulose-hemicellulose-lignin structure is disrupted so as to make cellulose more accessible to enzymes. During enzymatic hydrolysis the released cellulose is then converted into glucose. Therefore, the pretreatment methods are important as they can maximize the cellulose recovery from lignocellulosic materials into usable forms [5].

In most of the previous studies, oil palm biomass such as oil palm empty fruit bunch (OPEFB) were utilized for the production of high value-added products such as glucose and lactic acid [6–8]. To the best of our knowledge, studies on OPT are rather limited and it is worthy to explore its usage as an alternative substrate to replace the expensive refined sugars. In addition, it would significantly reduce the raw material cost. Besides, none of the pretreatment protocol is universal and economically viable for pretreatment of

* Corresponding author.
E-mail addresses: zki@unisel.edu.my (L.W. Lai), ani@cheme.utm.my, aniidris@utm.my (A. Idris).

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different cellulosic biomass. Hence, the present study attempts to pretreat OPT biomass using two different pretreatment methods (i.e. steam-alkali-chemical and microwave-alkali) and subsequently use it as the substrate for enzymatic saccharification. The objective was to evaluate the suitably of pretreatment methods for maximum cellulose recovery and hemicellulose and lignin reduction. Alkali was chosen as the pretreatment agent because it causes lesser sugar degradation [9]. The morphology, crystallinity and structural changes on OPT biomass were analyzed using FESEM, XRD and FT-IR, respectively.

2. Experimental

2.1. Raw materials

The OPT was a gift from Concept Renewable Energy Resources Sdn. Bhd., Johor, Malaysia. The pulverized OPT was sieved using Restuch's sieve shaker (AS 200 basis, Germany) and OPT with mesh size < no. 18 were collected and used throughout the experiment.

2.2. Chemical reagents and enzymes

All chemical reagents and enzymes were of analytical grade. The acetic acid (CH_3COOH), sodium hydroxide (NaOH) and sodium hypochlorite (NaClO) solutions were employed in pretreatment of OPT biomass. The chemicals such as ethanol (C_2H_5OH), toluene (C_7H_8), sodium chlorite ($NaClO_2$) and sulfuric acid (H_2SO_4) were used in the composition analyses. On the other hand, the 50 mM sodium citrate buffer was prepared by mixing citric acid ($C_6H_8O_7.H_2O$) and trisodium citrate dihydrate ($C_6H_5O_7Na_3.2H_2O$). The sodium azide (NaN_3) was employed in enzymatic saccharification step to inhibit the growth of microorganisms. The NaOH, 3,5-dinitrosalicylic acid ($C_7H_4N_2O_7$), sodium metabisulfite ($Na_2S_2O_5$), phenol (C_6H_6O), potassium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$) were mixed in the required proportions and employed as DNS reagent in glucose determination [10]. Two commercial enzyme solutions; celluclast 1.5 (cellulase) produced by *Tricoderma reesei* ATCC 26921 and Novozyme 188 (cellobiase) from *Aspergillus niger* were used in the enzymatic saccharification. The activities of both cellulase and cellobiase enzymes were measured at 88.15 FPU.ml^{-1} and 201.6 CBU.ml^{-1}, respectively [11,12].

2.3. Pretreatment protocol

2.3.1. Microwave-alkali (Mw-A) pretreatment

Briefly, the 2.5 M NaOH aqueous solution was used in microwave-alkali pretreatment to pretreat the OPT biomass at solid to liquid ratio of 1:20 [13]. The Sineo microwave (MAS-II, China) was preset to its operating conditions at 700 W, 80 °C and 60 min. Upon completion, the slurry was first cooled to room temperature and subsequently filtered through a Whatman filter paper, no 3. The filter cake was washed under running tap water (4 × 1000 ml), followed by distilled water (4 × 500 ml). The treated residues were then dried at 60 °C and used for subsequent analysis.

2.3.2. Steam-alkali-chemical (SAC) pretreatment

200 ml of 2.5 M NaOH was poured into a beaker containing 20.0 g of oil palm trunk biomass. The mixture was then brought to an ALP's autoclave (CL-32LDP, Japan) at temperature, 121 °C; time, 15 min and pressure, 0.12 MPa. After autoclaving, the slurry was left to cool down. The steam-alkali treated OPT was washed with tap water (4 × 1000 ml) and followed by distilled water (4 × 500 ml). In a second step, the filtrate was mixed with 200 ml NaClO. Consequently, the pH of mixture was measured using bench top pH meter (Thermo-scientific: Orion 2-star, Singapore) and adjusted to acidic condition (pH 3.5) using CH_3COOH [14]. The solution was then filtered-off and acid-treated OPT sample was further washed using tap water (4 × 1000 ml), followed by distilled water (4 × 500 ml). The SAC-treated oil palm trunk biomass was then dried at 60 °C and kept for subsequent experiments.

2.4. Compositional analysis

The raw and pretreated OPT were then tested for its composition. All the procedures were conducted in duplicates. The relative percentage difference (RPD) was set less than 5% to ensure the reproducible of attained results; revealing that the quality of the sample analysis was reliable [15]. The Soxhlet extraction on OPT biomass was performed by mixing both C_2H_5OH and C_7H_8 in the ratio of 1:1 as reported by TAPPI (1997) test method [16]. The H_2SO_4 acid determination of Klason's lignin content and quantification of delignified holocellulose residue by $NaClO_2$ in OPT were conducted accordingly [17,18]. The α-cellulose amount was determined using TAPPI (2009) test method [19]. Meanwhile, the hemicellulose amount of OPT was calculated as the difference between holocellulose and cellulose [18].

2.5. Morphology analyses

2.5.1. Field emission scanning electron microscopy (FESEM) images

FESEM analysis of raw and different pretreated OPT were carried out with Jeol's instrument (JSM-6071F, USA) at 5.0 kV. All samples were sputter coated with a thin layer of gold before imaging at 500× magnification.

2.5.2. Fourier Transform Infrared (FTIR) analysis

The spectra of raw and pretreated OPT biomass were determined using FT-IR spectroscopy (Pelkin Elmer 2000, USA). The changes in functional groups for all samples in this study were recorded between the wavenumber of 4000–370 cm^{-1} [20].

2.5.3. X-ray diffractometer (XRD) measurement

The crystallinity indices of raw and pretreated OPT samples were measured using Rigaku X-ray Diffractometer (D/Max 2500, Japan). Sample was first packed tightly in a rectangular glass cell with a dimension of 15 × 10 mm and thickness 1.5 mm. Radiation used was Cu Kα (λ = 1.54 Å) at 40 kV and 30 mA. The grade range was between 5 and 40° with a step size of 0.05°. The cellulose crystallinity is determined using Equation (1) [21]:

$$\mathrm{CrI}\ (\%) = [(I_{002} - I_{18^\circ})/I_{002}] \times 100 \quad (1)$$

where CrI, is the crystalline index; I_{002}, the maximum intensity of the (002) lattice diffraction and I_{18°, is the intensity diffraction at 18°.

2.6. Enzymatic saccharification study

The Mw-A pretreated OPT biomass with higher cellulose content as determined in Section 2.4 was selected and used in the enzymatic saccharification study. The reducing sugar concentration was represented with glucose equivalent [12]. It was used as an indicator for the effectiveness of enzymatic hydrolysis. The factors influencing enzymatic saccharification such as enzyme ratio, pH, temperature, substrate loading on glucose production were investigated. The enzymatic hydrolysate was withdrawn at intervals of 1, 2, 3, 4, 5, 24 and 48 h. The sample was directly mixed with DNS reagent in equal volumes (1:1) and it was then heated at 100 °C in the thermo shaker (Biosan 100, Korea) to cease the enzyme activity.