



Characterization of Empty Fruit Bunch Treated with Ionic Liquid Prior to Enzymatic Delignification

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Abstract

The technological utility of enzymes for delignification can be increased by using ionic liquid to open more accessible surface area for biomass transformation into bio-based products. The present paper demonstrates application of ionic liquid (IL) [emim][DEP] 1-ethyl-3-methylimidazolium-diethyl phosphate for empty fruit bunch (EFB) pretreatment process followed by enzymatic delignification by using Laccase. It was found that [emim][DEP] increased the performance of the enzyme laccase and hence higher cellulose rich materials, whereas also reduced the lignin content in the EFB. The lowest lignin content obtained from IL-laccase treated EFB was approximately 17.92%, lower than the lignin content in the untreated EFB. Both treated and untreated EFB were characterized in chemical and physical properties by using scanning electron microscope (SEM), fourier transform infrared (FTIR), and thermogravimetric analysis (TGA/DTG) to observe the changes resulted from the pretreatment.

Keywords: biomass pretreatment, cellulose, enzymatic delignification, ionic liquid

Abstrak

Penggunaan enzim untuk proses delignifikasi biomass dapat ditingkatkan dengan menggunakan cairan ionik (*ionic liquid*) untuk menyediakan substrat dengan luas permukaan yang dapat lebih mudah diakses oleh enzim mengkonversi biomass menjadi produk biomaterial. Penelitian ini menggunakan cairan ionik [emim][DEP] 1-ethyl-3-methylimidazolium-diethyl phosphate untuk pretreatment tandan kosong kelapa sawit (TKS) diikuti dengan proses enzimatik dengan menggunakan *Laccase* sp. Ditemukan bahwa [emim][DEP] dapat meningkatkan kinerja enzim dalam mendegradasi biomass komponen, dan menghasilkan TKS dengan kandungan lignin yang lebih rendah dan meningkatkan kandungan serat selulosa. Kadar lignin terendah diperoleh sebesar 17,92% setelah proses dengan cairan ionik diikuti dengan enzimatik. Fisikal karakteristik dilakukan dengan menggunakan scanning electron microscope (SEM), fourier transform infra-red (FTIR) dan thermogravimetric analysis (TGA) untuk mengamati perubahan pada TKS sebelum dan setelah perlakuan yang diberikan.

Kata kunci:biomass pretreatment, cairan ionik, delignifikasi, enzimatik, sellulosa

1. Introduction

Empty fruit bunch (EFB) is one of solid waste produced from palm oil industry. Oil palm tree is one of the most well-known and extensively cultivated plant families. Based on the preliminary experiment, EFB contains holocellulose about 49.06% and lignin about 25.33% (See Table 1).

EFB shows high content of holocellulose, which makes this biomass as a promising feedstock for biomaterial and biofuel products. However, the complexity in the

biomass structure created recalcitrance to any chemicals or enzymatic processes. Lignocellulose is mainly composed of the rigid semi-crystalline polysaccharide cellulose, the amorphous multicomponent polysaccharide hemicellulose, and the crosslinked aromatic polymer lignin, as a primary constituents of plant cell walls, in which cellulose fibers are embedded into entangled matrix consisting of lignin and hemicellulose, forming a tight and compact structure. These inherent properties of lignocellulosic materials make them resistant to the chemicals and enzymes.

Thus, pretreatment process needs to be taken at first, the aim of this pretreatment is to change these complex properties of lignocellulosic materials by breaking the lignin chain and the crystalline structure of cellulose simultaneously increasing the porosity of the cellulose rigid structure. Briefly, the mechanism of pretreatment can affect the physical properties of biomass such as a degree of polymerisation, a crystallinity structure and surface area of the solid substrate.

Ionic liquids (ILs), a potentially attractive 'green' recyclable alternative to environmentally harmful organic solvents, have been increasingly exploited as solvents and/or (co)solvent and/or reagent in a wide range of applications including pretreatment of lignocellulosic biomass (Sun *et al.*, 2011; Mora-Pale *et al.*, 2011; Moniruzzaman and Ono, 2012). Moreover, this IL provides many desirable properties such as low toxicity, low corrosiveness, low melting point ($< -20^{\circ}\text{C}$) and low viscosity (10 pa at 80°C) (Moniruzzaman and Ono, 2012).

Many experiments have been conducted using ILs to dissolve wood or lignocellulosic biomass at different conditions and with the combination of other processes, such as microwave radiation (Ha *et al.*, 2011), alkali pretreatment (Lan *et al.*, 2011) or followed by enzymatic delignification (Moniruzzaman dan Ono, 2012). ILs pretreatment are able to reduce the degree of polymerization of cellulose rich-material and leading to enhance the enzymatic delignification efficiency of wood biomass (Moniruzzaman and Ono, 2012; Moniruzzaman *et al.*, 2013).

The extensively explored of numerous studies focused on the dissolution of natural polymer especially cellulose in ILs as solvents, encourages this work to study more about the ability of IL to separate the cellulose and lignin in the EFB and to enhance the enzymatic delignification process with ultimate goal to obtain cellulose rich fibers with minimum structural alteration. EFB samples (treated and untreated) were characterized using SEM, FTIR and TGA analysis to see the effect of pretreatment to the physical structures of the sample and the chemical characterizations were analyzed using standard method as described in the methodology.

2. Method

2.1. Materials

Empty fruit bunch (EFB) from oil palm plantation FELCRA (Malaysia), was grinded and sieved to obtain particle size in the range of 0.25 - 0.5 mm). Ionic liquid, 1-ethyl-3-methylimidazolium-diethyl phosphate [enim][DEP], Commercial laccase (51639-56, $\geq 10\mu\text{g}/\text{mg}$) from *Trametes sp.* and 1-hydroxybenzotriazole (HBT) from Sigma (St. Louis, MO, USA) and all other reagents used in the experiments are analytical grade.

2.2. Dissolution of EFB in Ionic Liquid and Enzymatic Delignification

Ionic liquid was put into a 50 mL round-bottom flask with magnetic stirrer at 80°C for 1 h. EFB was added into ionic liquid (IL) with a ratio 10:1 (IL : Sample w/w). After cooling process, water:acetone mixture (1:1 v/v) was added into biomass-IL mixture and stirred for 20 min to separate the solid materials from dissolved IL and lignin. Then, the treated sample was washed with distilled water and put in the oven drying for overnight. For enzymatic delignification process, the treated sample (after IL treatment at 80°C for 1 hr) was placed into conical flask and added with sodium acetate (100 mM, pH 4.5) (ca. 5 wt.% biomass), Laccase ($\geq 10\mu\text{g}/\text{mg}$), and 1-hydroxybenzotriazole (HBT) (1.5 wt.% of biomass) was added as a mediator and stirred at 50°C . After 24 h, 0.1 M sodium hydroxide (NaOH) was added into the mixture and stirred for 1 h to extract lignin from the IL-enzyme treated EFB. The mixture was then filtered and washed with distilled water until pH 7 under mild vacuum prior to oven drying.

2.3. Characterization of Untreated and Treated Materials

Concisely, the holocellulose (α -cellulose and hemicellulose) determination was performed by using acidified sodium chlorite solution at 70°C at 1 h. In addition, α -cellulose was treated with 17.5% sodium hydroxide and 10% acetic acid. The difference values between holocellulose and α -cellulose gave hemicellulose value of the samples. The lignin content of the sample was analyzed according to ASTM D 1106-96 (Klason Lignin). The surfaces of samples were photographed by Scanning Electron Microscope (SEM) (TM 3030, Hitachi Ltd., Tokyo, Japan). Thermogravimetric analysis (TGA-Q50, TA instrument, USA) was performed to compare the degradation characteristics and thermal stability of the treated and untreated samples, by heating 5 mg of

sample in a platinum pan at a rate 10°C/min in a nitrogen atmosphere. Fourier Transform Infrared Spectroscopy (Thermo Nicolet IS10, USA) was conducted to determine any chemical changes occurring in the biomass sample during the pretreatment process.

3. Results and Discussion

3.1. Chemical and Physical Characterization of EFB

The chemical composition study of untreated and treated EFB is summarized in Table 1 as dry weight basis. It was observed that all regenerated cellulose materials have a considerably lower lignin content compared to corresponding original EFB. The lowest content of lignin obtained from EFB treated with [emim][DEP] prior to enzymatic delignification with laccase was approximately 17.92% (entry 3), lower than the original EFB lignin content. The efficiency of IL could be correlated with the anion and cation size as well as hydrogen bond basicity.

Anion from IL will disrupt the free hydroxyl group in the lignocellulosic material, whilst the cation will interrupt the hydroxyl oxygen atom, thus disrupt its three dimensional network. Consequently, hemicellulose and lignin is dissolved (Mäki-Arvela *et al.*, 2010). Furthermore, as shown in Table 1, compared with untreated EFB the cellulose content of treated EFB with IL prior to enzymatic delignification is higher because of the removal of hemicellulose, lignin, and soluble extractives during the treatment process.

3.2. The Morphology of EFB

Fig. 1 depicts SEM images of untreated and

treated EFB at which taken at 200 μm with magnification 500. The untreated EFB showed intact morphology, compact, ordered and rigid fibril structure because of the lignin coating on cellulose fibers (Fig.1), while IL-laccase treated EFB showed loose, disordered, and curly structure. This swelling may be a result of breaking inter-intramolecular hydrogen bonding caused by phosphate anion from [emim][DEP] during the pretreatment, which led to open more accessible area for enzyme laccase to degrade lignin.

3.3. Surface Composition of EFB analyzed by FTIR

The FTIR spectra of treated and untreated EFB are shown in Fig. 2. As observed in the Figure, the dominant peaks at 3346 cm^{-1} (O-H stretch) and 2892 cm^{-1} (C-H stretch) represent the aliphatic moieties and the prominent peaks at 1709 - 1735 cm^{-1} attribute to polysaccharides (Labbe *et al.*, 2005). There were subtle difference spectra between the untreated and treated EFB, particularly in the finger print region at 1508 - 1588 cm^{-1} and 1248 cm^{-1} (Faix, 1991; Fengel & Ludwig, 1991; Isroi *et al.*, 2012), which characterized the C=O aromatic skeletal for lignin's IR spectra in the untreated EFB, the bands were found disappeared in the treated EFB with ionic liquid prior to laccase treatment.

The dissipation of an IR absorbance of lignin in the treated EFB indicated the removal of some lignin during the treatment. This is consistent with the chemical composition study (see Table 1), in which the lignin percentage was obtained lower for samples with ionic liquid followed by laccase treatment compared to untreated EFB.

Table 1. Chemical composition of untreated and treated sample

Biomass component (% dry basis)	Empty Fruit Bunch (EFB)			
	Untreated	[emim] ^b [DEP]	[emim] [DEP]+Laccase	Laccase ^d
Alpha-cellulose	49.06 $\pm$ 1.53	53.28 $\pm$ 3.91	59.09 $\pm$ 4.67	49.43 $\pm$ 3.48
Hemicellulose	22.83 $\pm$ 1.16	18.91 $\pm$ 6.98	15.23 $\pm$ 3.00	19.55 $\pm$ 6.16
Lignin	25.33 $\pm$ 1.41	19.86 $\pm$ 1.32	17.92 $\pm$ 1.61	22.89 $\pm$ 6.07

^a The data represent the average of three experiments with standard deviation

^b Ionic liquid pretreatment condition IL : Biomass ratio 10:1 (w/w), 80°C for 1 h

^c Laccase treatment with sodium acetate buffer and HBT, at 50°C for 24 h

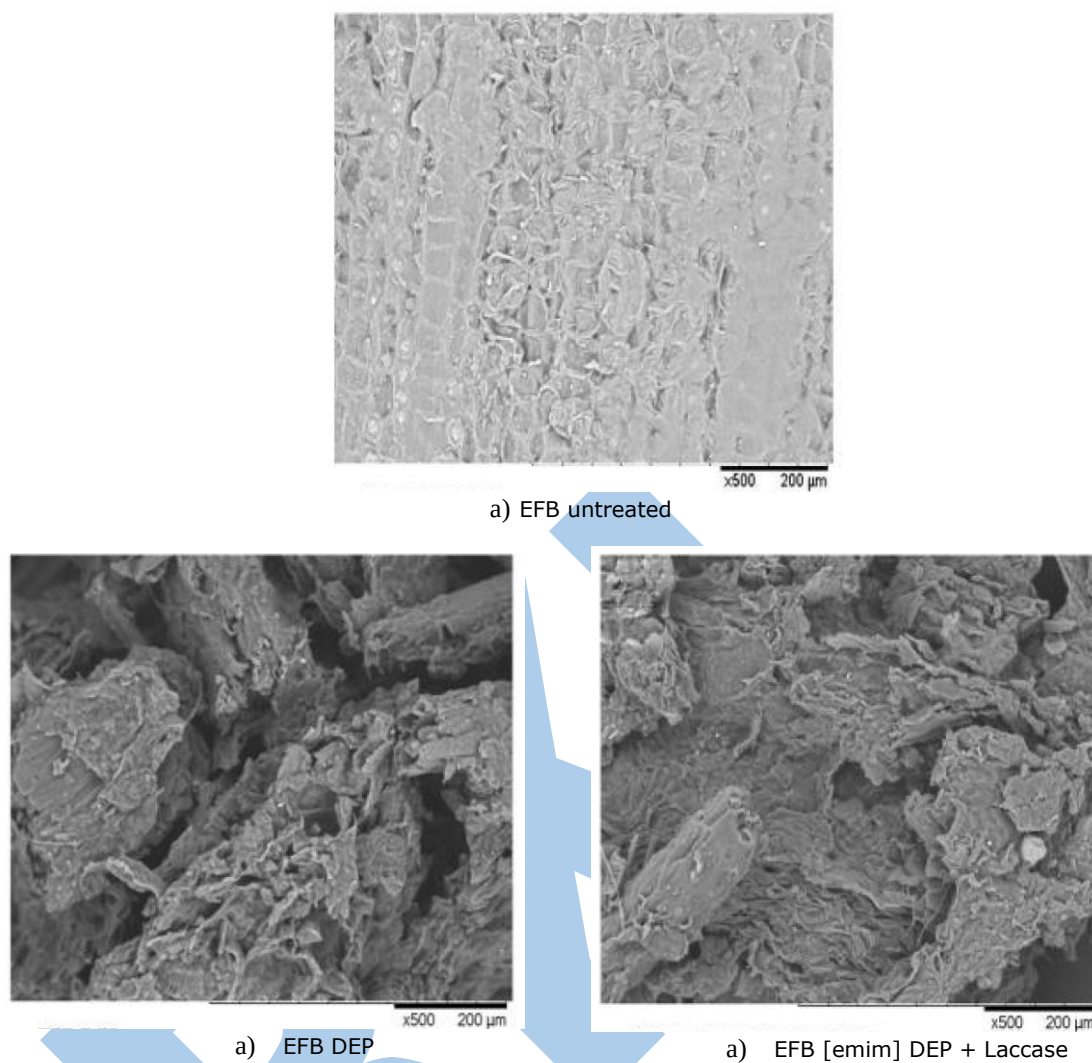


Figure 1. SEM images of untreated and treated EFB

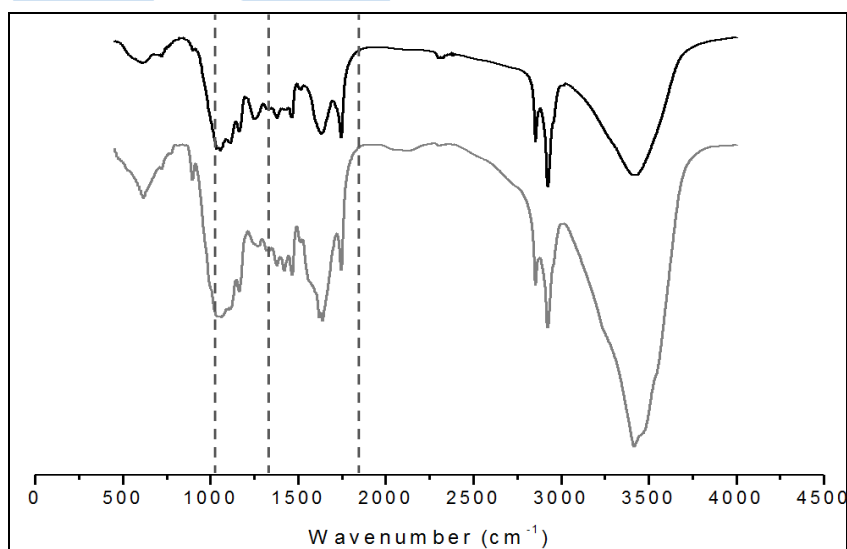


Figure 2. FTIR Spectra of EFB; — untreated, — treated with EFB [emim]DEP + Laccase

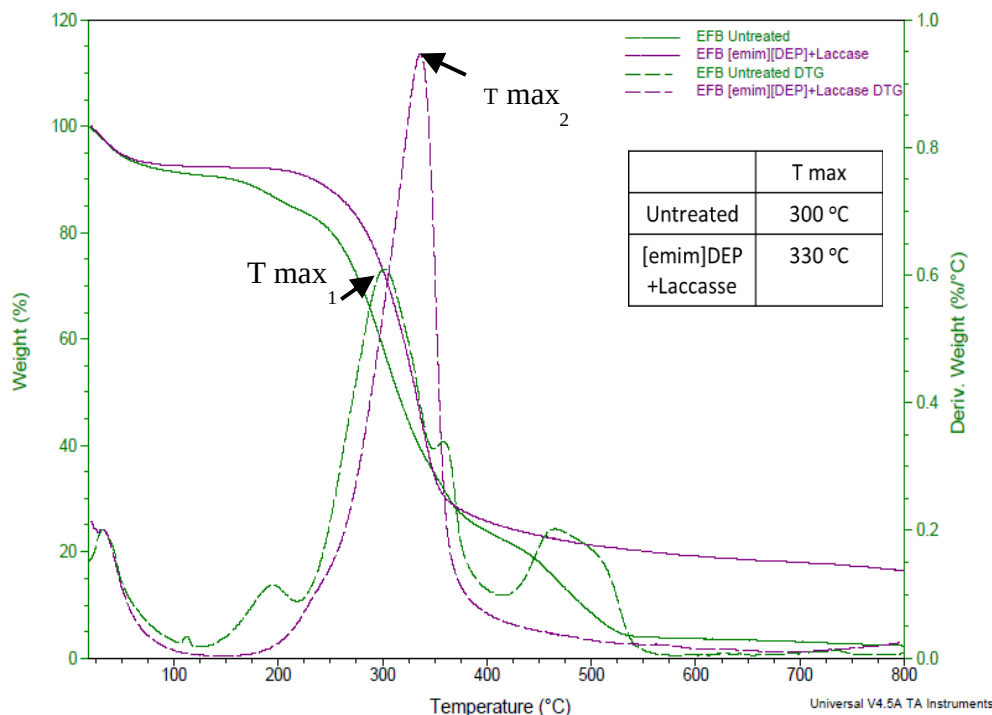


Figure 3. DTG/TGA curve for untreated and treated EFB

3.4. Thermal Stability of EFB

Thermogravimetric measures the change of biomass weight at a specific heating rate, because the physical and chemical reactions of sample when heated, showed the characteristics of the materials. EFB thermal decomposition curves for untreated and treated with ionic liquid are shown in Fig.3. The DTG data of untreated EFB show one dominant peak with a peak value (T_{max}) of 300°C, whilst EFB treated with ionic liquid followed by laccase treatment showed temperature peak at T_{max} 330°C, which was higher than that the untreated EFB.

The value of T_{max} represents the maximum decomposition rate occurs at certain temperature, in order to gauge the impact of thermal ability after ionic liquid pretreatment of biomass samples. This results implied that the thermal stability for treated EFB was more improved than that the original biomass, because of the transformation of cellulose I structure into cellulose II after the pretreatment which was reported had better thermal stability (Zhang *et al.*, 2014).

4. Conclusion

In summary, the delignification of Empty fruit bunch (EFB) with IL [emim][DEP] resulted in significantly reducing of lignin content up to 17.92% for IL-laccase

treated EFB, lower than that the untreated EFB. SEM images displayed a loose and disordered structure of EFB after ionic liquid and laccase treatment. FTIR analysis revealing some peaks of lignin were disappeared in the regenerated EFB, in which indicated typical of lignin peaks. The thermal stability of regenerated EFB was notably increased after the treatment, because of the transformation of cellulose I to cellulose II. Therefore, [emim][DEP] was a promising solvent for biomass delignification and able to enhanced the enzymatic process.

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