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Recovery of High Purity Lignin and Digestible Cellulose from Oil Palm Empty Fruit Bunch Using Low Acid-Catalyzed Organosolv Pretreatment

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Abstract: The lignocellulosic residue from the palm oil industry, oil palm empty fruit bunch (OPEFB), represents a challenge to both producing industries and environment due to its disposal difficulties. Alternatively, OPEFB can be used for the production of valuable products if pretreatment methods, which overcome OPEFB recalcitrance and allow tailored valorization of all its carbohydrates and lignin, are developed. Specifically, high-value applications for lignin, to increase its contribution to the feasibility of lignocellulosic biorefineries, demand high-purity fractions. In this study, acid-catalyzed organosolv using ethanol as a solvent was used for the recovery of high-purity lignin and digestible cellulose. Factors including catalyst type and its concentration, temperature, retention time, and solid-to-liquid (S/L) ratio were found to influence lignin purity and recovery. At the best conditions (0.07% H_2SO_4, 210 °C, 90 min, and S/L ratio of 1:10), a lignin purity and recovery of 70.6 ± 4.9% and 64.94 ± 1.09%, respectively, were obtained in addition to the glucan-rich fraction. The glucan-rich fraction showed 94.06 ± 4.71% digestibility within 18 h at an enzyme loading of 30 filter paper units (FPU) /g glucan. Therefore, ethanol organosolv can be used for fractionating OPEFB into three high-quality fractions (glucan, lignin, and hemicellulosic compounds) for further tailored biorefining using low acid concentrations. Especially, the use of ethanol opens the possibility for integration of 1st and 2nd generation ethanol benefiting from the separation of high-purity lignin.

Keywords: oil palm empty fruit bunch; lignin recovery; lignin purity; digestible cellulose; organosolv pretreatment

1. Introduction

As a result of being one of the largest crude palm oil producers in the world, Indonesia produces around 37 million tons of oil palm empty fruit bunch (OPEFB) yearly [1]. Indonesian oil palm plants are located and concentrated in some areas. For instance, more than 50% of the OPEFB is produced in 5 regions in Sumatra [2]. OPEFB accumulation can cause environmental problems including the proliferation of disease-causing pests and microorganisms, the requirement of extensive land for burying, and gas emissions originated from its combustion [3,4]. Therefore, handling and valorization of OPEFB are crucial, and at a starting point, the existence of areas with higher production of palm oil can be used as an advantage for valorization by decreasing transportation costs [2].

Being a lignocellulosic material, OPEFB is mainly composed of cellulose, hemicellulose, and lignin that account to 24–65 wt%, 21–34 wt%, and 14–31 wt% of the material, respectively [5]. The holocellulosic (cellulose and hemicellulose) fragments can be enzymatically converted into simple sugars [6] for further conversion into value-added products. However, the recalcitrance of OPEFB hinders easy access to its carbohydrate polymers. In general, enzymatic digestibility of lignocellulosic materials is limited by a number of factors such as the presence of lignin, cellulose crystallinity, degree of polymerization, acetyl groups bound to hemicellulose, surface area, and biomass particle size [7,8]. Hence, pretreatment is needed to open the lignocellulosic structure of OPEFB and have access to sugar polymers for further valorization.

Pretreatment of lignocelluloses can be performed by mechanical, chemical, enzymatic, and biological processes, or by a combination of these [9]. Chemical pretreatment is a strong and effective pretreatment to improve the digestibility of lignocellulosic biomass, applying acids, bases, or other catalysts such as hydrogen peroxide and ozone [10], with dissimilar end-results. For instance, in acid pretreatment, deconstruction of the lignocellulosic structure is carried out mainly through dissolution of hemicellulose, leaving lignin in the solid fraction together with cellulose [11]. The remaining lignin will interfere with the following enzymatic hydrolysis of cellulose. Alkali pretreatment can easily break the lignin bonds and enhance the solubilization of the polymer [12]. In this method, lignin is effectively removed; however, the lignin dissolved in the liquid is difficult to recover. Ozone pretreatment is safer than alkaline and acid pretreatments and leads to efficient lignin removal. Nonetheless, lignin recovery remains a hurdle and ozone generation is very expensive [13]. Hydrogen peroxide pretreatment is efficient towards the removal of lignin and xylan [14], but it is very toxic for the environment. Therefore, none of the pretreatment methods described can efficiently recover the lignin from the lignocellulose.

Lignin has normally been considered as a barrier to properly access carbohydrate polymers. As a low-value stream in lignocellulosic biorefineries, lignin is normally used for heat and power generation through combustion [15]. However, as a result of feasibility concerns of lignocellulosic biorefineries and increasing range of high-value applications for lignin, higher attention has been devoted to the full valorization of lignocellulose-derived polymers. In fact, high purity lignin can be utilized for the production of value-added products such as resin, flavor compounds, and nanofibers with antioxidant activity [16–18]. Hence, pretreatment methods that enable both efficient lignin removal from lignocelluloses and easy recovery into a high-purity lignin fraction, can positively contribute to the feasibility of lignocellulosic biorefineries.

The use of organic solvent (organosolv) for pretreatment is a promising strategy. The organic solvent is able to extract lignin and hydrolyses the hemicellulose [19]. A high-purity cellulose with only minor degradation and a high proportion of its amorphous phase, which is susceptible to enzymatic hydrolysis, can be recovered [20]. The extracted lignin can be further precipitated by dilution with water and recovered as a solid product, while hemicellulosic sugars remain in the liquid stream [19]. The organic solvent can easily be recovered using evaporation or distillation processes.

There are several parameters affecting the success of the fractionation process, especially delignification, during organosolv pretreatment such as solvent type and concentration, catalyst type and amount, temperature, retention time, and solid to liquid ratio (S/L ratio) [21]. Ethanol is a solvent that is frequently used for organosolv pretreatment of lignocellulosic biomass due to its low price, good solubility of lignin, lower toxicity compared to other alcohol-based solvents, its miscibility with water, and ease of recovery [22]. Moreover, the production of ethanol using sugars and starch-rich substrates is an industrially mature technology well distributed throughout the world; ca. 29,100 million gallons were produced in 2019 [23]. In the organosolv pretreatment, acids and bases can be added as catalysts to increase the delignification rate, whereas comparatively lower delignification rates (≤60%) are normally obtained during non-catalyzed organosolv pretreatment of lignocellulosic biomass [24,25]. Mineral acids have high reactivity and efficiency and sulfuric acid is the most studied catalyst for ethanol-organosolv pretreatment [26,27]. Higher S/L ratio is favorable because a smaller amount of solvent is employed and a better balance can be found among energy needed to carry out the pretreatment step and the amount of processed material. Overall, an organosolv pretreatment strategy that ensures high S/L ratio, minimum addition of acid and solvent, and optimum temperature and retention time, while attaining fractions that are rich in high-quality glucan, lignin, and hemicellulosic compounds, are of interest for the valorization of lignocellulosic materials.

The research landscape on organosolv pretreatment is characterized by a wide range of substrates and pretreatment conditions investigated, but by a lack of systematic studies to reveal unequivocally substrate-tailored pretreatment systems [28]. For instance, various organosolv strategies have been used for the pretreatment of OPEFB, as a result of extensive research on the development of efficient biorefinery systems for its valorization. These included the use of bisulfite, a mixture of acetic acid and ammonia, or ethylene glycol as solvents, where studies on ethanol organosolv pretreatment of OPEFB are scarce in literature [29–34]. Moreover, information about the influence of pretreatment parameters on lignin recovery and purity following organosolv pretreatment is still scarce in the literature, and it is common to all lignocellulosic substrates studied [28]. A recent study has shown that organosolv pretreatment conditions influence the recovery and purity of lignin from oat husks [35]. Therefore, the aim of the present study was to study the effect of acid-catalyzed ethanol organosolv pretreatment on the delignification of OPEFB. A range of parameters was studied in organosolv pretreatment of OPEFB, namely, acid type and concentration, temperature, retention time, and S/L ratio. Emphasis was given to lignin purity and lignin recovery as well as the digestibility of the glucan-rich fraction. The optimization steps carried out in this study led to high lignin purity and recovery. In addition, digestible glucan-rich fraction which has high glucan purity and recovery was obtained. The results obtained demonstrate the possibility to use very low acid concentration for deconstruction of OPEFB into high-quality fractions.

2. Materials and Methods

2.1. Materials

Oil palm empty palm fruit bunch (OPEFB) was obtained from a palm oil industry in Medan, Indonesia. It was sun-dried to achieve 7% moisture content. The dried OPEFB was then milled using a cutting mill (Retsch SM 100, Haan, Germany) using a screen with a pore size of 300 µm and resulted in the following particle size distribution: 44.24% of >500 µm; 17.96% of 250–500 µm; 23.81% of 100–250 µm; and 13.99% of 63–125 µm. The composition of OPEFB is presented in Table 1. The enzyme cocktail Cellic®® Ctec3 (Novozymes, Bagsværd, Denmark), with a cellulase activity of 222 filter paper units (FPU) /mL, was used for the hydrolysis of a mixture of glucan- and hemicellulosic compounds-rich fraction. The chemicals used were sulfuric acid, D-glucose, L-arabinose, D-maltose, D-xylose, and D-galactose from Sigma-Aldrich, and pure ethanol (100%) and glacial acetic acid from Scharlau.

Table 1. Composition of oil palm empty palm fruit bunch (OPEFB).

Component.	OPEFB (% Dry Weight)
Lignin	21.77 ± 0.27
Glucan	40.09 ± 0.01
Hemicellulose	23.94 ± 0.02
Protein *	4.18 ± 0.51
Ash	3.72 ± 0.07
Others	6.30

* A nitrogen-to-protein conversion of 6.25 was used.

2.2. Organosolv Pretreatment

Oil palm empty fruit bunch (OPEFB), solvent ethanol 50% (v/v), and acid catalysts were added to 150 mL stainless steel tubular reactors (Swagelok, El Paso, TX, USA). The raw material and solvent were added according to the required solid loading. The reactors were sealed and placed into an oil bath (Julabo, Seelbach Germany) at specific temperature and retention time. After the desired retention time, the reactors were removed from the oil bath and quenched directly in an ice bath. The mixture was filtered using a 250 µm sieve for separation of the solid (glucan-rich) and the liquor (solvent, lignin-rich, and hemicellulosic compounds-rich) fractions after cooling down. The solid was washed using 28.3 mL of solvent/g of dry sample. The solvent was being collected and added to the liquor. To induce precipitation of lignin, 56.6 mL of water/g of dry sample was added to the liquor which was then centrifuged at 3360× *g* for 5 min to separate the lignin from the solvent and hemicellulosic compounds-rich fraction. Then, lignin-rich fraction, glucan-rich fraction, and hemicellulosic compounds-rich fractions were kept in the refrigerator at 4 °C until use. Series of pretreatments were carried out by varying four parameters, namely, acid type and concentration, temperature, retention time, and S/L ratio. To study the effect of catalyst, two strategies were used: (a) sulfuric acid and acetic acid were added to the solvent to reach the pH of 3; and (b) acetic acid was added in the equal amount as that of sulfuric acid added to reach the pH of 3. The pretreatment temperatures studied were 180, 210, and 220 °C; the retention times were 30, 60, 90, and 120 min; and the S/L ratios were 1:20, 1:10, and 1:5. The experiments were carried out using a one factor-at-time strategy.

2.3. Enzymatic Hydrolysis

The glucan-rich and hemicellulosic compounds-rich fractions obtained at the best conditions found in the study were mixed and evaporated using a rotary evaporator (LABO ROTA 20, Heidolph, Schwabach, Germany) at 110 °C, 40 rpm, and at a vacuum pressure of 100 mPa. The concentrated slurry had a glucan loading of 1.37% (w/v) and 1.85% (w/v) of total solid. Cellic®® Ctec3 enzyme solution was prepared by 10× dilution with ultra-pure water followed by sterile filtration using disposable disc filters with a pore size of 0.2 µm (GVS, Findlay, OH, USA). Diluted enzyme solution was added to the slurry based on the enzyme activity and glucan content (10, 15, 20, and 30 FPU/g glucan). Enzymatic hydrolysis was performed in 20 mL Erlenmeyer flasks, containing 10 mL of slurry adjusted to pH 5.2, that were incubated in a water-bath at 50 °C and shaking at 125 rpm for 24 h. Samples of 1.5 mL were withdrawn at 18 h and 24 h for chromatographic analysis. The percentage of glucan hydrolysis was then calculated based on the ratio between the amount of glucose released after hydrolysis and the theoretical maximum. Untreated OPEFB and evaporated hemicellulosic compounds-rich fraction were also used for enzymatic hydrolysis for comparison purposes.

2.4. Analytical Methods

The moisture content of untreated OPEFB and of the glucan-rich and lignin-rich fractions, to determine recovery yields, was quantified through sample drying in an oven at 70 °C until constant weight. The total solids of the evaporated mixture of glucan-rich and hemicellulosic compounds-rich fractions were determined according to Sluiter et al., (2008) [36]. The lignin-rich fraction, glucan-rich fraction, and untreated OPEFB were also analyzed for carbohydrates, lignin, and ash according to the methods described by Sluiter et al., (2008) [37], in order to determine the purity of the fractions. The percentage of lignin recovery was calculated using the following Equation (1)

$$(\%)\ \text{Lignin recovery} = \frac{\text{Lop} \times (\%)\ \text{lignin purity}}{\text{Lf}} \times 100\% \qquad (1)$$

Lop = weight of lignin-rich fraction obtained after organosolv pretreatment; Lf = weight of lignin on OPEFB feed

Derived sugars from the acid treatment of samples for compositional analysis and glucose released during enzymatic hydrolysis were measured using High Performance Liquid Chromatography (HPLC) (Waters, Milford, MA, USA). The system was equipped with a hydrogen-based column (Aminex HPX-87P, Bio-Rad, Milford, MA, USA) operating at 60 °C and using 0.6 mL/min of 5 mM H_2SO_4 as the eluent. A refractive index (RI) detector (Waters 2414) was used to quantify the compounds.

The crystallinity of the untreated and pretreated OPEFB was analyzed using a Fourier Transform Infrared (FTIR) spectrometer using Nicolet OMNIC 4.1 software (Impact 410 iS10, Nicolet Instrument Corp., Madison, WI, USA). The spectral data were obtained with an average of 64 scans and resolution of 4 cm^{-1}, in the range of 400–4000 cm^{-1}. The total crystallinity index was calculated by the absorbance ratio of wavenumbers 1428 cm^{-1} and 897 cm^{-1} [38].

The nitrogen content of OPEFB was analyzed using the Kjeldahl method according to Mahboubi et al., (2017) [39] and the crude protein was obtained by using a nitrogen-to-protein conversion factor of 6.25.

2.5. Statistical Analysis

All experiments and analyses were carried out in duplicate. All intervals and error bars reported represent two times the standard deviation. The data acquired were statistically analyzed using MINITAB®® 17 (Minitab Ltd., Coventry, UK). A general linear model with a confidence interval of 95% was applied for the analysis of variance (ANOVA); statistical differences were identified at p-value <0.05. To have a better understanding of the extent of differences between results obtained, pairwise comparisons according to the Bonferroni test were performed.

3. Results

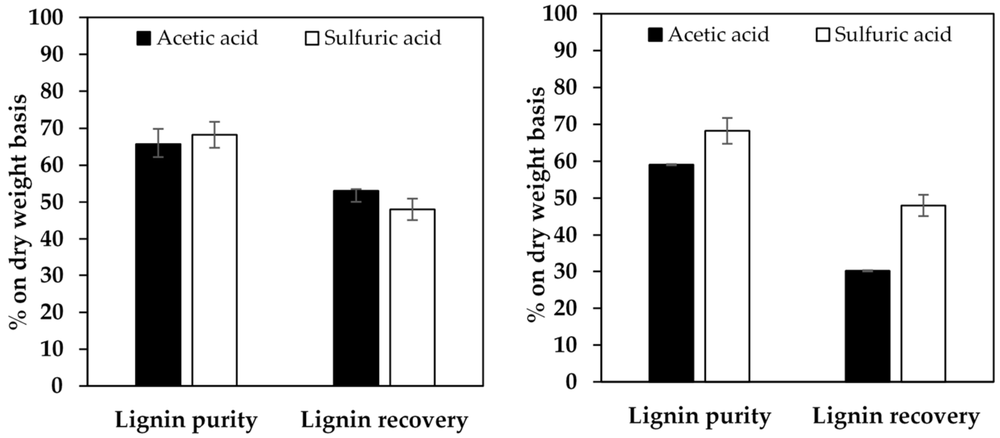
Oil palm empty fruit bunch was pretreated using acid-catalyzed ethanol organosolv to achieve deconstruction into three high-quality streams, namely, glucan-rich, lignin-rich, and hemicellulosic compounds-rich fractions. The effect of pretreatment factors, namely, acid catalyst type and concentration, temperature, retention time, and S/L ratio on lignin purity and recovery were studied. A mixture of evaporated glucan and hemicellulosic compounds-rich fractions obtained at the best condition of pretreatment was then enzymatically hydrolyzed in order to investigate the effect of organosolv pretreatment on glucan digestibility. The untreated OPEFB was also enzymatically hydrolyzed as a control.

3.1. Effect of Acid Catalyst

Catalyst Type and Concentration

In order to evaluate acetic acid and sulfuric acid as catalysts, ethanol organosolv pretreatment of OPEFB was carried out at 210 °C for 120 min with S/L ratio of 1:20. Both acids were added to the solvent until an initial pH of 3 was achieved. The amount of acetic acid added was 0.32 g acid/g substrate, while the use of sulfuric acid was much lower, i.e., 0.0013 g acid/g sample. The effect of catalyst type on lignin purity and recovery is presented in Figure 1a. The addition of acetic acid and sulfuric acid did not have a significant effect on the lignin purity, which were 65.70 ± 4.10 and 68.25 ± 3.50%, respectively, based on the dry weight. Lignin recovery from the two pretreatment conditions also showed no significant difference (53.02 ± 0.46% for acetic acid and 48 ± 2.91% for sulfuric acid). Since the amount of acetic acid added was higher than that of sulfuric acid, sulfuric acid is thus considered more efficient as a catalyst.

A further experiment was carried out where the amount of acetic acid added to the solvent was equal to the amount of sulfuric acid added to reach pH 3. The amount of both acids added was thus 0.0013 g/g sample. The final pH of the solvent with acetic acid was 4.66. Results from this experiment are presented in Figure 1b. Organosolv pretreatment with sulfuric acid showed better performance with significantly higher lignin purity (68.25 ± 3.50%) and lignin recovery (48.00 ± 2.91%) than those obtained when acetic acid was the used catalyst. The lignin purity of OPEFB treated with acetic acid as catalyst was lower by 9.17% while the recovery was lower by 17.85%. As sulfuric acid was more efficient regarding lignin purity and recovery, it was therefore employed for all subsequent pretreatment experiments.

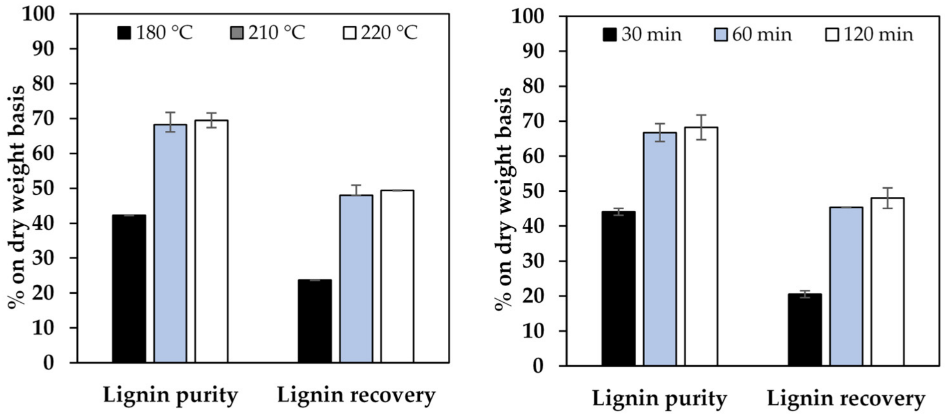


(**a**) (**b**)

Figure 1. Effect of (**a**) catalysts type (added in different concentration to reach pH 3) and (**b**) catalyst concentration (added in the same concentration, 0.13% (w/w)) on lignin purity (*p*-value = 0.444 and *p*-value = 0.035, respectively) and recovery (*p*-value = 0.076 and *p*-value = 0.007, respectively). Error bars represent ± 2× the standard deviation.

3.2. *Effect of Temperature and Retention Time*

Three different temperatures, namely, 180 °C, 210 °C, and 220 °C were employed for organosolv pretreatment of OPEFB for 120 min with S/L ratio of 1:20 and using sulfuric acid as catalyst. The lignin purity and recovery were significantly higher when the temperature of the pretreatment was increased from 180 °C to 210 °C (Figure 2a). However, when the temperature was 220 °C, no further improvement on lignin purity and recovery were observed. Hence, 210 °C was chosen for further pretreatment experiments.



(**a**) (**b**)

Figure 2. Effect of (**a**) temperature (for 120 min and using solid-to-liquid (S/L) ratio of 1:20) and (**b**) retention time (at temperature of 210 °C and S/L ratio of 1:20) on lignin purity (*p*-value = 0.001 and *p*-value = 0.001, respectively) and recovery (*p*-value = 0 and *p*-value = 0, respectively). Error bars represent ± 2× the standard deviation.

To investigate the effect of retention time, the pretreatment was conducted for 30, 60, and 120 min. Increasing the retention time from 30 to 60 min resulted in higher lignin purity (from 44.09 ± 0.41 to 66.73 ± 2.55) and recovery (from 20.52 ± 1.27 to 45.35 ± 0.00%) (Figure 2b). However, no significant improvements were observed by extending the pretreatment to 120 min.

3.3. Effect of S/L Ratio

The S/L ratio is the ratio of solid phase (biomass) and liquid phase (solvent plus water) in the organosolv pretreatment. In the first approach, different S/L ratios, namely, 1:20, 1:10, and 1:5, were applied for 60 min at 210 °C. The lignin purity obtained from pretreatment experiments at different S/L ratios of 1:20, 1:10, and 1:5 was 66.73 ± 2.55, 77.60 ± 2.80, and 80.26 ± 2.09%, respectively. Changing the S/L ratio from 1:20 to 1:10 increased the purity of lignin, however, no further improvement on lignin purity was achieved at S/L ratio of 1:5 (Figure 3a).

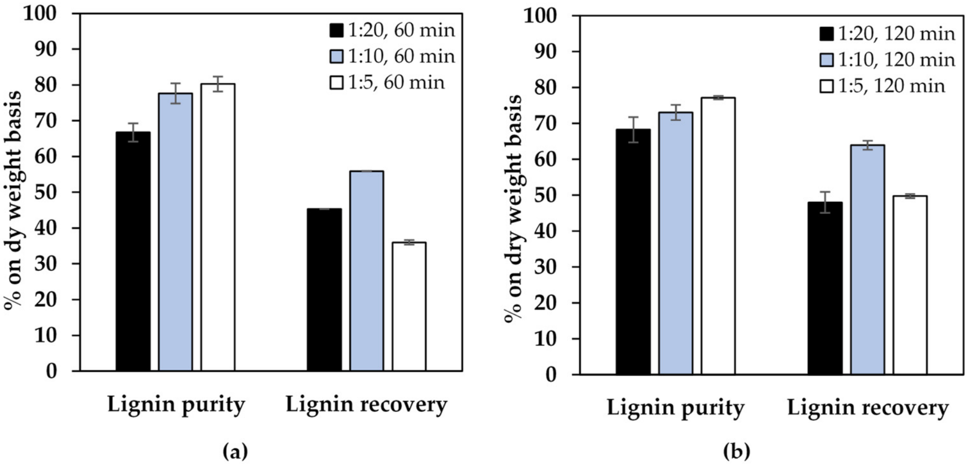


Figure 3. Effect of solid to liquid ratio and retention time of (**a**) 60 min and (**b**) 120 min (pretreatment at 210 °C) on lignin purity (p-value = 0.009 and p-value = 0, respectively) and recovery (p-value = 0 and p-value = 0.002, respectively). Error bars represent ± 2× the standard deviation.

However, such a trend was not observed for lignin recovery (Figure 3a), where a significant decrease was observed when the S/L ratio was changed from 1.10 to 1:5 (55.86 ± 0.06% vs. 35.99 ± 0.63%, respectively). Accordingly, the retention time was extended to 120 min (Figure 3b); similar trends were found for lignin purity and recovery although an overall increase in the later was observed. These results together with those obtained throughout the optimization approach, culminating in higher purity and recovery, indicate the interaction between S/L ratio and retention time which should be the focus of future research.

In a further optimization experiment, a retention time of 90 min was also investigated for pretreatment at an S/L ratio of 1:10. This retention time resulted in a significantly higher lignin recovery of 64.94 ± 1.09% than that obtained from the pretreatment at 60 min which was 55.85 ± 0.06%. A similar lignin recovery value of 63.94 ± 1.25% was obtained at 120 min (Figure 4); therefore, shorter retention times can be used while achieving similar recovery and purity of lignin. In summary, the best conditions found among those tested in this work, for the recovery and purity of lignin, include sulfuric acid as catalyst, temperature of 210 °C, retention time of 90 min, and S/L ratio of 1:10. In these conditions, lignin purity of 70.56 ± 4.48% and lignin recovery of 64.94 ± 1.09% were obtained.

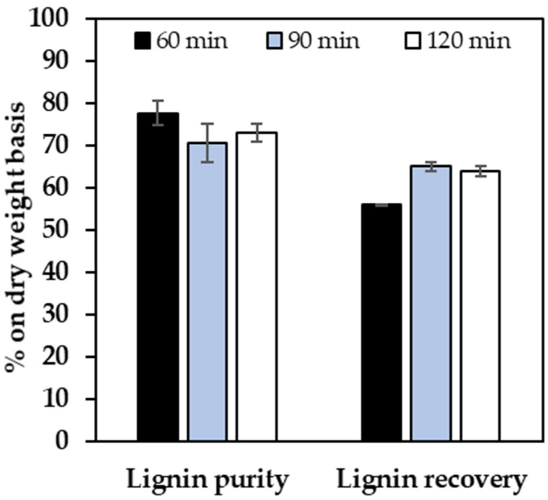


Figure 4. Effect of retention time at S/L ratio of 1:10 on lignin purity (p-value = 0.118) and recovery (p-value = 0.002). Error bars represent ± 2× the standard deviation.

Analysis of the composition of the lignin-rich fraction and the glucan-rich fraction, obtained under the best conditions mentioned, was carried out and the result is presented in Table 2. Based on the recovery of lignin in the glucan-rich fraction (around 10%), delignification of about 90% was obtained which shows some material loss during recovery through centrifugation.

Table 2. Composition of glucan-rich fraction and lignin-rich fraction obtained after acid-catalyzed organosolv pretreatment at 210 °C, for 90 min, and using an S/L ratio of 1:10.

Compound	Glucan-Rich Fraction	Lignin-Rich Fraction
Glucan	74.16 ± 0.52%	10.11 ± 0.08%
Glucan recovery	80.00 ± 0.02%	5.06 ± 0.08%
Lignin	5.09 ± 0.48%	70.56 ± 4.48%
Lignin recovery	10.44 ± 0.78%	64.94 ± 1.09%
Hemicellulose	8.24 ± 0.06%	7.79 ± 0.06%
Hemicellulose recovery	15.40 ± 0.11%	6.54 ± 0.16
Ash	3.23 ± 0.34%	1.87 ± 0.00%
Others	9.28%	9.67%

3.4. Crystallinity of Cellulose

The effect of organosolv pretreatment on the crystallinity of OPEFB was determined by calculating the total crystallinity index (TCI) value (absorbance ratio at wavenumbers 1248 and 897 cm^{-1}). Crystallinity index can be used to observe changes in cellulose digestibility after pretreatment. Glucan-rich fraction spectra showed a higher absorption band at 897 cm^{-1} and a lower absorption band at 1428 cm^{-1} (Figure 5). The result shows an increase in amorphous cellulose and a decrease in crystalline cellulose after pretreatment which indicates an increase in cellulose digestibility. The TCI value of the untreated OPEFB was 1.25 ± 0, whereas after pretreatment in the best conditions, i.e., 0.07% H_2SO_4, 210 °C, 90 min, and a solid-to-liquid ratio of 1:10, the TCI was 0.95 ± 0.03, representing a reduction of 24.48%.

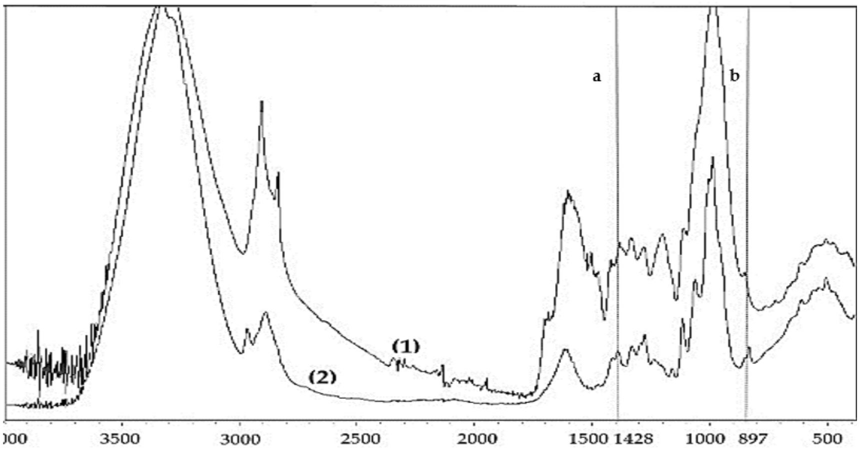
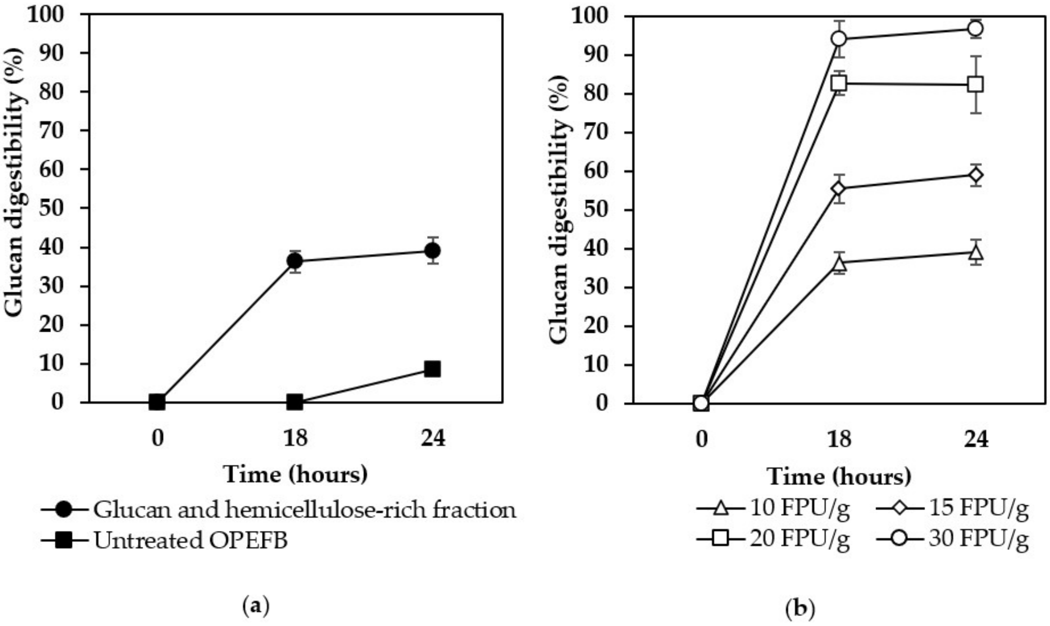


Figure 5. FTIR spectra of **(1)** untreated oil palm empty fruit bunches (OPEFB) and **(2)** glucan-rich fraction from the best organosolv pretreatment condition (0.07% H_2SO_4, 210 °C, 90 min, and solid-to-liquid ratio of 1:10). Bar **(a)** shows the absorbance at 1428 cm^{-1} whereas bar **(b)** shows the absorbance at 897 cm^{-1}.

3.5. Enzymatic Hydrolysis

Untreated OPEFB and a mixture of evaporated glucan-rich fraction and hemicellulosic compounds-rich fraction were digested using 10 FPU of Cellic®® Ctec3 enzyme per gram of glucan, for 24 h. The use of organosolv pretreatment led to an increase of 4.56-fold in glucan digestibility (Figure 6a). Enzymatic hydrolysis of evaporated hemicellulosic compounds-rich fraction resulted in 0.25 g/L glucose after 18 h. Since an enzyme loading of 10 FPU/g glucan could not achieve 100% glucan digestibility, higher enzyme loadings were used (Figure 6b). There was no difference on glucan digestibility between 18 h and 24 h of enzymatic hydrolysis. Therefore, enzymatic hydrolysis for 18 h resulted in glucan digestibility of 36.29 ± 2.84%, 55.45 ± 3.72%, 82.66 ± 3.09%, and 94.06 ± 4.71% at enzyme loading of 10, 15, 20, and 30 FPU/g glucan, respectively.



(**a**) (**b**)

Figure 6. Glucan digestibility of (**a**) untreated OPEFB and of a mixture of evaporated glucan-rich and hemicellulosic compounds-rich fractions at enzyme loading of 10 FPU/g glucan and (**b**) a mixture of evaporated glucan-rich and hemicellulosic compounds-rich fractions using different enzyme loadings. Error bars represent ± 2× the standard deviation.

4. Discussion

Organosolv pretreatment uses organic solvents to remove lignin and hemicellulose, leaving a high purity glucan-rich fraction after solid-liquid separation. The fine tuning of organosolv pretreatment can consider a manifold of parameters including the solvent type and its concentration, catalyst addition, catalyst type and its concentration, temperature, retention time, S/L ratio, biomass particle size, and pretreatment vessel design. Substantial research on organosolv pretreatment has been carried out using a wide range of organosolv systems, applied to a wide range of lignocellulosic materials. The emphasis has been on the optimization of the pretreatment system in order to recover a high-purity and highly digestible glucan fraction, while the effect of pretreatment parameters on the recovery and purity of lignin has been comparatively less investigated [28]. Moreover, research studies using acid-catalyzed ethanol organosolv for the pretreatment of OPEFB are scarce in the scientific literature.

In this work, we demonstrated that parameters including catalyst type and its concentration, temperature, retention time, and S/L ratio had an effect on the recovery and purity of lignin following ethanol organosolv pretreatment of OPEFB. Through a series of optimization steps, where factor interaction is hypothesized to take place, OPFEB was decomposed into glucan-, lignin-, and hemicellulosic compounds-rich fractions. A delignification of ca. 90% was obtained at the following conditions: 0.07% H_2SO_4 (g/g substrate), 50% ethanol, 210 °C, 90 min, and S/L ratio of 1:10. The purity and recovery of glucan was 74% and 80%, respectively, and the corresponding values for lignin were 71% and 65%, respectively.

Lignin is the most abundant aromatic polymer in nature. Lignin is composed by coniferyl alcohol and sinapyl alcohol, with a small amount of *p*-coumaryl alcohol. Currently, most of the lignin (in low-purity) is produced by Kraft pulping process which reaches 50 million tons/year as low-value residuals [40]. Low-quality lignin limits its range of applications. Lignin produced through organosolv pretreatment of lignocellulose materials can be of high-purity, widening the range of potential applications. High-purity lignin can be converted into various polymers such as surfactants, plasticizers, superabsorbent gels and hydrogels, coating, and stabilizing agents [41–43]. Lignin also has the potential to be utilized as raw material for nanofiber with antioxidant capacity, resins, and vanillin synthesis [16–18]. The compositional analysis of the glucan-rich fractions revealed that loss of lignin takes place during the precipitation and centrifugation steps. This can be related to the intrinsic limitations of the recovery methods used or to the chemical characteristics of the resulting lignin. It has been reported that the dissolved lignin is polydispersed and, therefore, the used solvent for precipitation might be specific for certain lignin fractions. Reduced efficiency of water as anti-solvent has been reported for lower-molecular weight lignins with high contents of methoxyl and phenolic hydroxyl groups [44]. Therefore, establishing relationships among organosolv pretreatment parameters, lignin molecular weight and surface characteristics, and lignin recovery yields, need to be considered in future studies. Furthermore, lignin surface and compositional characteristics will influence the end-application and, consequently, influence the economic contribution of lignin to lignocellulosic biorefineries.

Among the organic solvents employed for organosolv pretreatment of lignocellulosic materials, ethanol is the most studied solvent due to its low price, good solubility of lignin, lower toxicity compared to other alcohol-based solvents, its miscibility with water, and ease of recovery [45]. Furthermore, the use of ethanol as solvent opens the possibility for integration of 1st and 2nd generation ethanol plants. Such strategy has been proposed as a result of techno-economic bottlenecks that still need to be overcome for commercialization of lignocellulosic biorefineries [46]. Accordingly, the glucan-rich fraction could be directed to yeast fermentation into ethanol, while the hemicellulosic compounds-rich fraction could be added to the yeast fermentation left-overs, following distillation, for e.g., biogas production through anaerobic digestion. Another strategy would be to take advantage of the capacity of filamentous fungi to consume pentose sugars so additional ethanol and fungal biomass for feed applications could be produced [47]. The ethanol as an end-product or as a pretreatment solvent could be recirculated through the system through already installed distillation columns and evaporators. Such integration strategy contributes to a needed holistic approach for cost-effective organosolv pretreatment processes, where optimization of pretreatment parameters leading to efficient material fractionation, need to be coupled with efficient fraction separation and solvent recycling methods [48]. By leading to lower energy consumption, the use of distillation is preferable to the use of evaporation for solvent recycling in systems containing water/low-boiling point organic solvent such as ethanol [48]. Reasonably, the extent of dilution of the black liquor will influence the energy consumption during distillation; therefore, studies on optimization of the amount of water added to induce lignin precipitation are also of relevance.

In this study, the crystallinity of glucan-rich fraction was 24.48% lower than that of the raw OPEFB. A decrease in crystallinity was also observed for pretreated Loblolly pine using 65% (v/v) ethanol with 1.1% (w/w) H_2SO_4 as the catalyst at 170 °C for 1 h [49]. Following OPEFB delignification and decreased crystallinity, 94.06 ± 4.71% glucan digestibility was achieved with an enzyme loading of 30 FPU/g within 18 h. Previous studies showed that high digestibility yield (>75%) of glucan-rich fraction from organosolv pretreatment of lignocellulosic material was obtained with enzyme loading higher than 10 FPU/g for 42–78 h of hydrolysis [50–53]. In addition to its use for ethanol production, glucan-rich fraction has also been proposed for production of acetone-butanol-ethanol (ABE) solvent, bio succinic acid, and fat-rich biomass [28].

A catalyst is usually added during organosolv pretreatment. The main effect in the addition of a catalyst is an increase in the rate and extent at which hydrolysis of hemicellulose and the cleavage of lignin-lignin bonds (α- and β-aryl ether linkages) occur [54]. Two types of acid catalysts i.e., acetic acid and sulfuric acid were employed in this study. The lignin recovery and purity of pretreatment using sulfuric acid as catalyst were significantly higher than those achieved when acetic acid was added at the same amount. To achieve similar lignin purity and recovery, about 270 times more acetic acid was needed. Even though acetic acid is more environmentally friendly, the high amount of acetic acid added can become problematic in the further utilization of lignocellulose fractions, that is, through microbial conversion where acetic acid can act as an inhibitor [55]. The higher lignin purity and its recovery after pretreatment with sulfuric acid as the catalyst is more likely due to the higher reactivity and efficiency of sulfuric acid on breaking the lignin-carbohydrate and lignin-lignin bonds than acetic acid [21]. The result from this study is in agreement with previous research by de la Torre et al., (2013) [56] who studied lignin recovery yields with different catalysts including acetic acid and sulfuric acid. Wheat straw was pretreated with 50% ethanol as a solvent and 0.001 N of each catalyst was tested for 30 min pulping. The organosolv pretreatment using acetic acid as catalyst led to 51% lignin recovery, whereas pretreatment using sulfuric acid led to 61% lignin recovery. Therefore, the results of this study support the need for alternative and effective catalysts. The catalyst should have a comparatively lower environmental footprint than that of sulfuric acid, to be used in organosolv pretreatment systems, a research gap previously identified [28].

The observations made on lignin purity and its recovery while varying the temperature and retention time agree with those reported by other studies. In this study, when the temperature was increased from 180 °C to 210 °C, lignin purity and recovery were increased by ca. 62% and 103%, respectively. This result is in agreement with an existing strong relationship between lignin solubility and temperature [57]. High delignification (>85%) was achieved during organosolv pretreatment of Silver birch wood chips and Norway spruce using ethanol as solvent and 1% sulfuric acid as catalyst at 200 °C [51,58]. The purity of the lignin from organosolv pretreatment of Silver birch wood chips was higher than that obtained in this work though, which was 96% [58]. A study by Goh et al., (2011) [59] reported 52% of lignin recovery from organosolv pretreatment of OPEFB using ethanol 65% (v/v) as solvent and 1.63% of sulfuric acid as the catalyst at a temperature of 190 °C. Generally, to obtain a better delignification rate, longer retention times are required. Results from this study showed that the use of retention time of 30 min was only able to recover 20% of lignin with a purity of less than 50%. When the pretreatment time was prolonged to 60 min, 45% of lignin was able to be recovered with 66% purity. A retention time of 60 min was reported to be necessary in order to obtain high delignification

(≥80%) on organosolv pretreatment of mixed sawmill and beechwood [60,61]. A study by Alio et al., (2019) [60] showed that 57% of lignin was recovered from organosolv pretreatment sawdust mixture of softwood species using sulfuric acid as a catalyst for 60 min. Furthermore, a retention time >60 min was needed in order to recover >30% of lignin during sulfuric acid-catalyzed organosolv pretreatment of OPEFB [59].

The S/L ratio is another key parameter for the commercialization of organosolv pretreatment. Higher S/L ratio is more favorable due to higher concentrations of glucan, hemicellulosic compounds, and lignin per unit volume. The higher concentration of substrate per volume leads concomitantly to the reduction of the amount of catalyst and solvent used per gram of substrate. However, the S/L ratio plays a role in organosolv pretreatment performance by affecting the contact between biomass and solvent. An S/L ratio higher than 1:10 was found to be detrimental to lignin purity and its recovery. Among research works applying organosolv systems for the pretreatment of lignocellulosic materials, such S/L ratio predominates [38]. The use in this study of an S/L ratio of 1:10 represented a concentration of 0.07% H_2SO_4 (gram acid per gram of substrate), which is almost 10× lower than that normally used for organosolv pretreatment [38], while attaining similar delignification yields together with high lignin recovery and purity. For instance, a study carried out by Goh et al., (2011) [59] showed that in order to recover 52% of the lignin from organosolv pretreatment of OPEFB using ethanol 65% as solvent at 190 °C for 75 min, 1.63% sulfuric acid was needed as a catalyst. Such a lower amount of acid used can have impacts on corrosion potential, economic feasibility, environmental footprint, and on the application of edible fungal biomass as feed ingredients. Altogether, the results of this work show the potential of acid-catalyzed ethanol organosolv pretreatment for fractionation of OPEFB and achieved a set of conditions leading to high lignin recovery and purity and digestible glucan. Moreover, the research work demonstrates the possibility to use much lower concentrations of acid with potential economic and environmental impacts. However, the system still can benefit from more environmentally-friendly catalysts and further system optimization that allow higher solid loading to be used, lower water usage during lignin precipitation, and clear relationships between lignin characteristics and end-application. Combining these optimization and characterization strategies with integration of OPEFB in 1st generation ethanol plants can create a beneficial biorefinery system for a such readily available lignocellulosic material in Indonesia, and in other countries such as Malaysia, the second largest worldwide producer of palm oil.

5. Conclusions

The purity and the recovery of both glucan and lignin from OPEFB could be obtained through a series of sequential steps of ethanol organosolv pretreatment. Lignin purity and recovery of ca. 71% and 65%, respectively, were obtained, opening the potential for higher-value applications. Furthermore, high-purity glucan fraction was also obtained that showed ca. 94% digestibility within 18 h of enzymatic treatment. The study shows that high delignification of OPEFB (of ca. 90%) and consequent recovery of high-purity lignin- and glucan-rich fractions can be obtained at remarkably lower acid concentration (0.07%) in comparison to previous studies within the area as result of an increase in the solid loading used.

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