Characteristic of oil palm empty fruit bunch after ethanol-organosolv pretreatment catalyzed by acid

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Abstract. The increasing growth of palm oil industry results in an increase in the accumulation of waste generated from the industry, such as oil palm empty fruit bunches (OPEFB). OPEFB is a good source of renewable raw materials if a pretreatment process can overcome the recalcitrance of OPEFB and allows the fractionation of all carbohydrates and lignin. In this work, the effectiveness of organosolv pretreatment to deconstruct OPEFB and to recover high purity of lignin and glucan was studied. The pretreatment experiments were carried out at 210 °C for 90 minutes with a solid to liquid ratio of 1/10. Ethanol 50% (v/v) was used as solvent. The effect of H2SO4 0.07% (w/w) as catalyst was also studied. The results showed that organosolv pretreatment with the addition of acid increased the purity of glucan and lignin by 36 and 5%, respectively. A total of 65% lignin was recovered, which was 3.6 times higher than lignin from pretreatment without acid catalyst. After organosolv pretreatment with an acid catalyst, the crystallinity index (CRI) decreased by 54%, while the decrease in CRI was only 14.4% after pretreatment without an acid catalyst. The water retention value of the pretreated OPEFB using acid catalyst increased by 8%. It can be concluded that organosolv pretreatment using ethanol as a solvent with the addition of acid catalyst succeeded in fractionating OPEFB with high purity and reducing recalcitrant OPEFB.

1. Introduction
Palm oil is one of the most widely produced vegetable oils to meet the world's vegetable oil needs. Palm oil is attractive because it has the lowest production cost among other vegetable oils [1], while its yield is relatively higher [2]. Indonesia is one of the largest palm oil producers in the world, with the production of 43 million tons of crude palm oil (CPO) in 2019 [3]. However, because of the large production of palm oil, a large amount of solid waste such as oil palm empty fruit bunch (OPEFB) is generated. For every 1 kg of CPO produced, 1.3 kg of OPEFB is generated [4]. Hence, approximately 55.9 million tons of OPFEB were produced in Indonesia in 2019. The high content of cellulose (24-65 wt%), hemicellulose (21-34 wt%), and lignin (14-31 wt%) [5] makes OPEFB as a potential raw material to produce value-added products that have the potential as substitutes for oil-derived fuels and chemicals [6].

Lignocellulosic-based biorefinery is currently facing economic feasibility problems, causing research attention to be directed to the overall utilization of all lignocellulosic components, including lignin [7]. Generally, lignin is removed during pretreatment because it acts as a barrier, which blocks
enzyme to access carbohydrate polymers. When lignin is recovered in high purity, a wide range of applications are available such as nanocomposites, UV-adsorbent, micro-capsulation, and food flavoring agents [8]. Therefore, a pretreatment method that can efficiently fractionate each of lignocellulosic polymer and recover high-purity lignin is important to achieve a feasible lignocellulosic-based biorefinery.

Most pretreatment methods do not separate lignin at high-purity, instead lignin remains in a slurry and mixed with other lignocellulosic-derived compounds [9]. On the contrary, organosolv pretreatment could separate lignocellulosic polymers into high-quality fractions [10]. The solvent used in the organosolv pretreatment dissolves hemicellulose and lignin, separating them from the solid glucan-rich fraction. Lignin is then precipitated by dilution with water and isolated from the hemicellulose-rich fraction [11]. Organosolv pretreatment factors such as solvent type and its concentration, temperature, and retention time affect the purity and recovery of lignin [7]. In organosolv pretreatment, ethanol is the most frequently used alcohol solvent because it is relatively cheap, good in lignin solubility, and easy to be recovered [12]. Pretreatment of ethanol-based organosolv can be carried out by adding a catalyst, most of which is an acid catalyst [13]. Acid catalysts are most widely used because of their high reactivity and efficiency [14, 15]. Therefore, the effect of acid catalyst on the recovery and purity of lignin- and glucan-rich fractions as well as on their structural changes was investigated in this study. In addition, since acid catalyst hydrolyzes carbohydrates, the changes in glucan digestibility were also studied.

2. Materials and method

2.1. Oil palm empty fruit bunch
Oil palm empty fruit bunch (OPEFB) was obtained from a palm oil industry in Medan, North Sumatra, Indonesia. The OPEFB was sun-dried until the moisture content reached 7%. The sun-dried OPEFB was then milled using a rotor mill (Retsch SM 100, Germany) equipped with a 300 μm pore-sized screen. Milled OPEFB has 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 OPEFB consisted of glucan 40.09±0.01%, hemicellulose 23.94±0.02%, lignin 21.77±0.27%, protein 4.18±0.51%, and ash 3.72±0.07% [16].

2.2. Organosolv pretreatment
OPEFB and solvent ethanol 50% (v/v) were put into a 150 ml stainless steel tubular reactor (Swagelok, USA) with a solid to liquid (S/L) ratio of 1:10. To study the effect of an acid catalyst, 0.07% (g/g substrate) of sulfuric acid was added. The tubular reactors were then sealed and incubated in an oil bath (Julabo, Germany) at 210 °C for 90 min. After 90 min of incubation, the reactors were removed from the oil bath and placed directly in an ice bath to cool down. The mixture inside 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. The glucan-rich fraction was then washed using 28.3 ml of solvent/g of dry sample. To precipitate the lignin in the liquor part, 56.6 ml of water/g of the dry sample was added. Subsequently, the liquor was centrifuged at 3360 g for 5 minutes to separate the lignin from the solvent and hemicellulosic compounds-rich fraction. Then the lignin-rich fraction, glucan-rich fraction, and hemicellulosic compounds-rich fractions were kept in the refrigerator at 4 °C until use.

2.3. Analytical methods
The moisture content of untreated OPEFB, glucan-rich, and lignin-rich fractions were determined by thermogravimetric approach. Samples were dried in an oven at 70 °C until a constant weight was reached, and the weight losses due to evaporation of moisture were recorded. To determine the purity of the fractions, the carbohydrates, lignin, and ash contents of untreated OPEFB, glucan-rich, and lignin-rich fractions were analyzed according to the methods presented by Sluiter et al. [17]. Derived sugars from acid hydrolysis of samples for compositional analysis were measured using high performance liquid chromatography (HPLC) (Waters, USA), which was equipped with a hydrogen-
based column (Aminex HPX-87P, Bio-Rad). The HPLC was operated at 60 °C and used 0.6 mL/min of 5 mM H₂SO₄ as the eluent. A refractive index (RI) detector (Waters 2414) was used to qualify and quantify the compounds. The percentage of lignin recovery was then determined based on the calculation by Mondylakista et al. [16].

The structure of the lignin-rich fraction was studied using proton nuclear magnetic resonance (¹H NMR) with deuterated Dimethyl sulfoxide (DMSO) as the solvent. ¹H NMR spectra were recorded on a Bruker Avance III HD (700 MHz ¹H) equipped with a QCI cryoprobe. The temperature was set to 25 °C. The 1D spectra were collected with a repetition delay of 15 s, and the signal was accumulated 16 times.

Fourier Transform Infrared (FTIR) spectrometer (Impact 410 iS10, Nicolet Instrument Corp., Madison, U.S.A.) was used to analyze the crystallinity of the untreated and pretreated OPEFB. The spectra were obtained with an average of 64 scans and a resolution of 4 cm⁻¹, in the range of 400-4000 cm⁻¹. The process was controlled using Nicolet OMNIC 4.1 software. The total crystallinity index (TCI) was calculated by the ratio of absorbance values at wavenumbers 1428 cm⁻¹ and 897 cm⁻¹ [18].

The morphology of the untreated OPEFB dan glucan-rich fractions from organosolv pretreatment with and without the addition of acid catalyst was observed using scanning electron microscope (SEM), model Jeol JSM-6510LA (Tokyo, Japan). The SEM images were recorded using a voltage of 10 kV.

Water retention values (WRV) of the untreated and the pretreated OPEFB were measured according to the method described by Noori and Karimi [19]. An amount of 0.1 g (dry weight based) of each material was placed in a non-woven material bag and immersed for 1 hour in the distilled water. Then, the swollen samples were weighed and the WRV of the sample was measured as (W2−W1)/W1, in which W1 is the weight of the dry sample and W2 is the final weight of the swollen sample.

2.4. Statistical analysis
All the experiments and analyses were conducted in duplicate, with all intervals reported represent two times of the standard deviation. The data acquired were then statistically analyzed using MINITAB® 17 (Minitab Ltd., 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. Result and discussion
Oil palm empty fruit bunch was pretreated using ethanol as a solvent with and without the addition of sulfuric acid as catalyst. Organosolv pretreatment was conducted to deconstruct and fractionate OPEFB into three streams, namely, glucan-rich, lignin-rich, and hemicellulosic compounds-rich fractions. The effect of acid catalyst on the recovery and purity of glucan-rich and lignin-rich fractions were studied as well as the effect of acid catalyst on the structural changes of both fractions.

3.1. Effect of acid catalyst on the composition, recovery, and purity of glucan-rich and lignin-rich fractions
The composition of glucan-rich fraction and lignin-rich fraction after organosolv pretreatment of OPEFB with and without the addition of acid catalyst was analyzed and the results are presented in table 1. The lignin purity of OPEFB after organosolv pretreatment with and without the addition of acid catalyst were 70.56±4.48 and 67.09±1.68%, respectively. The lignin-rich fraction obtained from pretreatment without the addition of catalyst had lower purity because it contained higher impurities. The corresponding value for the lignin-rich fraction with the addition of catalyst was 5.95%. The lignin recovery from the pretreatment with the addition of an acid catalyst was 64.94±1.09%. Whereas, it was only 17.85±0.97% of lignin was able to be recovered from the pretreatment without acid catalyst.
During organosolv pretreatment, lignin dissolution occurs due to the cleavage of α- and β-aryl ether bonds [13]. Generally, α-aryl ether bonds are more easily disintegrated than β-aryl ether bonds. However, since β-aryl ether bond accounts for 40-65% of total linkages in lignin, its cleavage is important for greater delignification [20]. The cleavage of β-aryl ether bond is enhanced in acidic conditions [21]. Moreover, the addition of acid catalyst aids enhanced hydrolysis of lignin–hemicellulose bonds [13]. Therefore, the higher purity and recovery of lignin-rich fraction from pretreatment with acid catalyst could be a result of more intense cleavage of β-aryl ether bonds as well as lignin–hemicellulose bonds. Previous study by Hochegger et al. [22] reported that lignin recovered from organosolv pretreatment of European Larch using 75% (v/v) ethanol with the addition of 1.65% (w/w) of sulfuric acid was 2.4 times higher than the one without acid catalyst.

Furthermore, a glucan purity of 74.16±0.52% was obtained after organosolv pretreatment with an acid catalyst. Whereas without the addition of an acid catalyst, a lower glucan purity of 54.53±0.27% was obtained. The lower glucan purity was due to the higher percentage of impurities such as lignin and hemicellulose, which were 19.87 and 19.99%, respectively. In addition, 55% of total lignin was recovered in the glucan-rich fraction when an acid catalyst was not added. This means that only 45% of delignification was achieved. Meanwhile, the glucan-rich fraction from organosolv pretreatment with acid catalyst contained 10% of total lignin, indicating 90% of delignification. This result supports the fact that adding acid catalyst on organosolv pretreatment could enhance the fragmentation and dissolution of lignin. Other than lignin, more hemicellulose was left in the glucan-rich sample from pretreatment without acid catalyst, which was 51.20% of the total hemicellulose. Whereas with the addition of acid catalyst, only 15.40% of hemicellulose was left in the glucan-rich fraction. The reason for this could be that the addition of acid catalyst on organosolv pretreatment increases the severeness of the hydrolysis of glycosidic bonds in hemicellulose and cellulose [21]. More severe of the disruption of glycosidic bonds when acid catalyst was added to the organosolv pretreatment might also explain the lower glucan recovery of 80% compared to the one without acid catalyst, which was 83.60%. The results from this study are in accordance with previous results by Choi et al. [23] who reported higher lignin removal of 49% on glucan-rich fraction when 1% (w/w) sulfuric acid was added as a catalyst on organosolv pretreatment of *Eucalyptus pellita*. Moreover, almost 100% of the hemicellulose was also removed from the glucan-rich fraction.

### 3.2. Effect of acid catalyst on the structure of the recovered lignin

Proton NMR spectrometry was used to study the structure of lignin because it offers the possibility to estimate several types of structural elements in lignin. It is also a reliable tool for semi-quantification of the content of lignin functional groups, such as phenolic groups, aliphatic hydroxyl groups, and

| Compounds         | Lignin-rich fraction | Glucan-rich fraction |
|-------------------|----------------------|----------------------|
|                   | With acid catalyst*  | Without acid catalyst |
|                   |                      | With acid catalyst*  |
| Glucan (%)        | 10.11±0.08           | 19.72±0.94           | 74.16±0.52 | 54.53±0.27 |
| Glucan recovery (%)| 5.06±0.08            | 5.23±0.25            | 80.00±0.02 | 83.60±0.41 |
| Lignin (%)        | 70.56±4.48           | 67.09±1.68           | 5.09±0.48  | 19.87±0.48 |
| Lignin recovery (%)| 64.94±1.09           | 17.85±0.97           | 10.44±0.78 | 55.04±2.73 |
| Hemicellulose (%) | 7.79±0.06            | 5.95±0.03            | 8.24±0.06  | 19.99±0.36 |
| Hemicellulose recovery (%) | 6.54±0.16 | 2.65±0.01 | 15.40±0.11 | 51.20±0.92 |
| Ash (%)           | 1.87±0.00            | 5.80±2.53            | 3.23±0.34  | 2.88±0.17  |
| Others (%)        | 9.67                 | 1.44                 | 9.28       | 2.73       |

*The results are taken from Mondylaksita et al. [14]
methoxyl groups. The spectra results are shown in Figure 1. As shown in the figure, there was a slight difference in the intensity of aliphatic groups between the two samples.

![Figure 1. 1H NMR spectra of lignin-rich fraction from the pretreated OPEFB with and without the addition of acid catalyst.](image)

The lignin-rich fraction obtained from organosolv pretreatment with acid catalyst had a lower intensity of aliphatic groups compared to that of without acid catalyst. A model reaction of β-aryl ether bond cleavage on organosolv pretreatment with acid-catalyst proposed by McDonough [24] could explain this phenomenon. In this reaction, γ- hydroxymethyl group is released as formaldehyde and forms enol ether bond on the side chain of lignin, then the C-C aliphatic bond is broken and further decreases the number of aliphatic hydroxyl groups. These results are in accordance with the results from previous study by Hochegger et al. [22], who reported that after organosolv pretreatment on larch sawdust, there was a decrease in the aliphatic hydroxy groups of lignin when the acid catalyst was added. The results from 1H NMR together with the results from compositional analysis of lignin- and glucan-rich fractions reinforce the notion that the addition of acid catalyst enhances the delignification process through more intense cleavage of the β-aryl ether bonds.

3.3. Effect of acid catalyst on the crystallinity and the morphology of glucan-rich fraction

The crystallinity of cellulose of the untreated and the pretreated OPEFB was studied using FTIR. The IR spectra of the untreated OPEFB, the pretreated OPEFB with acid-catalyst, and the pretreated OPEFB without acid catalyst are presented in Figure 2. The 1428 and 897 cm\(^{-1}\) absorption bands were used to study the crystallinity changes. The band at 1428 cm\(^{-1}\) shows crystalline cellulose I, whereas the band at 897 cm\(^{-1}\) represents the cellulose II. The organosolv pretreatment with and without the addition of acid catalyst reduced the absorption band at 1428 cm\(^{-1}\) and increased the band at 897 cm\(^{-1}\). This corresponds to a reduction in crystalline cellulose I and an increase in amorphous cellulose II.
Figure 2. FTIR spectra of the untreated OPEFB and glucan-rich fraction from the pretreated OPEFB with and without the addition of acid catalyst.

The effect of acid catalyst on the crystallinity of the pretreated OPEFB was determined by calculating the total crystallinity index (TCI) value. Total crystallinity index was calculated from the absorbance ratio at $A_{1428}/A_{897}$ (table 2). When acid catalyst was added to the organosolv pretreatment, the TCI value was reduced by 54%, whereas without the addition of acid catalyst, the TCI value decreased only 14.4%. These results are in agreement with a previous study by Meng et al. [25], who reported a 3% decrease in the crystallinity index of poplar after organosolv pretreatment using 60% (v/v) of ethanol with the addition of 1.25% (w/w) of sulfuric acid as catalyst. The decrease in crystallinity index after organosolv pretreatment with acid catalyst indicates that acid catalyst was capable to disrupt the para-crystalline part of cellulose [25].

Table 2. The total crystallinity index (TCI) of the untreated OPEFB and glucan-rich fraction from the pretreated OPEFB with and without the addition of acid catalyst.

| Materials                                  | TCI       |
|--------------------------------------------|-----------|
| Untreated OPEFB                            | 1.25±0.00$^a$ |
| Glucan-rich fraction without acid catalyst | 1.07±0.03$^b$ |
| Glucan-rich fraction with acid catalyst    | 0.58±0.01$^c$ |

* Means ±SD with different letters are significantly different ($\alpha=0.05$)

The surface morphology of the untreated OPEFB and the glucan-rich fractions from the pretreated OPEFB was observed by scanning electron microscopy (SEM). The SEM micrographs of the untreated OPEFB and the glucan-rich fractions from organosolv pretreatment with and without acid catalyst are presented in Figure 3. The untreated OPEFB fibers had a rigid appearance with a refined surface (Figure 3a). Some physical changes such as a less stiff appearance and rougher fiber surface can be seen after organosolv pretreatment without the addition of acid catalyst (Figure 3b). Whereas on the glucan-rich fraction of the OPEFB after pretreatment with the addition of acid catalyst, not only rougher surface but also the existence of pores on the fibers surface were observed (Figure 3c). Changes in the fiber surface occurred due to the removal of lignin and hemicellulose, which act as surface impurities. The formation of pores indicates a more severe disruption of the OPEFB fiber structures [26]. The micrographs show that organosolv pretreatment with the addition of acid catalyst...
was able to remove surface impurities and make the fiber surface rougher and more porous, which is important for increasing the digestibility of pretreated OPEFB. This result is in line with the FTIR results where the addition of acid catalyst reduced TCI value more severely than without the addition of acid catalyst.

**Figure 3.** Scanning electron micrographs (magnitude 5000x) of (a) untreated OPEFB (b) glucan-rich fraction without acid catalyst addition (c) glucan-rich fraction with acid catalyst addition.

### 3.4. Effect of acid catalyst on the swelling capacity of glucan-rich fraction

Water retention value, or also known as swelling capacity is usually used as an indicator that shows the surface accessibility of the cellulose structure to enzyme’s attack [19]. The principle is based on the fact that if water cannot enter the pores of the cellulose, then the enzymes cannot access the cellulose, either. WRV has a linear relationship with the hydrolysis rate of substrates [27, 28]. Moreover, the results are comparable to the results obtained from more advanced methods such as NMR and Simons’ staining [29, 30].

The results from this study showed that WRV of the organosolv-pretreated OPEFB without the addition of acid catalyst was not significantly different from the untreated OPEFB (table 3). However, when acid catalyst was added, the WRV of the pretreated OPEFB increased by 90%. The possible explanation is because organosolv pretreatment with the addition of acid catalyst gave a lower lignin content than the one without acid catalyst. A previous study by Ju et al. [31] reported that the presence of lignin in biomass hinders the swelling of cellulose fiber, which results in the decrease of WRV. The results of WRVs obtained in this study are also in line with the results from the FTIR assay. Both results indicate the increase of the surface accessibility or digestibility of the glucan after organosolv pretreatment with the addition of acid catalyst.

**Table 3.** Water retention value of the untreated OPEFB and glucan-rich fraction from organosolv-pretreated OPEFB with and without the addition of acid catalyst.

| Materials                        | WVR (g/g) |
|----------------------------------|-----------|
| Untreated OPEFB                  | 0.60±0.12b|
| Glucan-rich fraction without catalyst | 0.82±0.11b|
| Glucan-rich fraction with catalyst | 1.13±0.08a|

* * Means ±SD with different letters are significantly different (α= 0.05)

### 4. Conclusion

The results of this study showed that the addition of acid catalyst in organosolv pretreatment of OPEFB increased the recovery and purity of lignin. The high recovery and purity of lignin were due to the more severe cleavage of β-aryl ether bonds when the acid catalyst was added. Furthermore, high-purity glucan could be recovered after the pretreatment with an acid catalyst. Analysis of crystallinity,
morphology, and water swelling capacity confirmed that the glucan recovered after organosolv pretreatment with acid catalyst had improved digestibility.

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Acknowledgments
The authors would like to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia under Pendidikan Magister menuju Doktor untuk Sarjana Unggul (PMDSU) Batch III (grant number 2971/UN1.DITLIT/DIT-LIT/LT/2019) and the Swedish Research Council (VR) for the financial support. The authors are also grateful for the technical support with NMR from Dr Diana Bernin (Chalmers University of Technology, Sweden).