The effects of FeCl3 concentration on hydrothermal pretreatment of oil palm fronds to enhance reducing sugar production

by Sunardi Sunardi

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The effects of $FeCl_3$ concentration on hydrothermal pretreatment of oil palm fronds to enhance reducing sugar production

Nurwahdah[1], Rodiansono[1], A Kamari[2], R Abdullah[3] and Sunardi[1,4]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru 70714, Indonesia
[2]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjung Malim, Perak, Malaysia
[3]Centre of Advanced Material and Energy Sciences, Universiti Brunei Darussalam, BE1410, Brunei Darussalam
[4]Wetland-Based Materials Research Center, Lambung Mangkurat University, Banjarbaru 70714, Indonesia

E-mail: sunardi@ulm.ac.id, masunardi@gmail.com

Abstract. The effects of $FeCl_3$ concentration on hydrothermal pretreatment of oil palm fronds (OPF) to enhance reducing sugar production have been systematically investigated for the first time. The hydrothermal pretreatment was carried out in autoclave reactor with Teflon at 190 °C for 30 min with various $FeCl_3$ concentrations of 0.075; 0.150; and 0.225 M. The residue from hydrothermal pretreatment (then noted as a substrate) was hydrolyzed by the enzyme (Cellulase Onozuka RS) in water bath shaker at 50 °C for 48 h. The amount of reducing sugar was analyzed by DNS (dinitrosalicylic acid) method using UV-visible spectroscopy. The reduced mass of substrate, change of color, alteration of pH of filtrate, and functional group analysis using Fourier Transform Infrared Spectroscopy (FTIR) results indicated the decomposition of OPF structures. The highest reducing sugar (3.800 g/L) of the substrate was obtained when the concentration of 0.225 M was used, which was higher than the pretreatment without $FeCl_3$ added (2.673 g/L). Overall, our study concludes that the hydrothermal pretreatment assisted by $FeCl_3$ can catalyze the decomposition of OPF structures to give the enzymes accessibility and enhance the reducing sugar production.

1. Introduction

Oil palm is one of the largest plantation commodity sectors in South Kalimantan, which produces large waste biomass, such as oil palm empty bunches and oil palm fronds (OPF) [1]. Oil palm fronds (OPF) contain 41.88% cellulose, 33.61% hemicellulose, and 20.65% lignin [2]. The highest cellulose of OPF biomass can further converted into any chemical feedstocks [3] such as reducing sugar (especially glucose), which can be fermented into second-generation bioethanol as the alternative energy of fossil fuels.

One of the processes for converting lignocellulose to second-generation bioethanol is the pretreatment process. The pretreatment process of lignocellulose is a critical step for removing lignin, reducing cellulose crystallinity, and increasing the porosity of the material due to complex cross-linking bonds between chemical components [4]. Pretreatment using a dilute strong acid solution has

the disadvantage of having the potential to produce side products such as furfural and hydroxy-methyl-furfural (HMF), which can inhibit the fermentation process and pollute the environment [5]. Some disadvantages of the pretreatment method in biology are: the low rate of hydrolysis is produced; most ligninolytic microorganisms consume not only lignin but also hemicellulose and cellulose; the pretreatment process is time-consuming; and the growth of microorganisms needs to be continuously controlled [6]. Therefore, the pretreatment method that is environmentally friendly, effective, highly efficient, and has lower energy consumption is needed to commercialize of bioethanol to reduce the total production costs.

Hydrothermal pretreatment continues to be developed to date because it is non-toxic, environmentally friendly, economical, and not corrosive [7]. Hydrothermal pretreatment without catalyst has been studied with a variety of lignocellulosic sources including wheat straw [8], corn cobs [9], palm leaves and fronds [10], hardwood and softwood [11], and oil palm fronds [12]. The use of chloride salt from the transition group as a catalyst in the pretreatment process of steam or microwave shows the higher efficiency of enzymatic hydrolysis. Moodley & Kana [13] reported that pretreatment with steam salt-alkali (SSA) using 1.73 M $ZnCl_2$, 1.36 M NaOH, and microwave salt-alkali (MSA) using 1.67 M $ZnCl_2$, 1.52 M NaOH at 400 W for 10 m was able to delignify lignin of 80.5 and 73% respectively. The advantage of pretreatment with SSA and MSA is that short pretreatment times on a large scale reduces energy consumption, thereby reducing production costs. Chloride salt from the transition group is non-polluting, very low toxicity, high catalytic activity, and less corrosive than acid [13, 14]. The fiber dissolved in 65% of the $ZnCl_2$ solution is capable of destroying large amounts of intra and intermolecular hydrogen bonds that cause destructions of the crystal regions, where cellulose molecules are separated from each other [40].

Kamireddy et al. [16] have conducted pretreatment of corn stover with the addition of chloride salt from the transition group such as $AlCl_3$ and $CuCl_2$ at the temperature of 160 °C, the concentration of 0.125 M, and the reaction time for 10 minutes which were compared with $FeCl_3$ and dilute sulfuric acid pretreatments at the same reaction conditions. On Kamireddy's study, pretreatment using $FeCl_3$ shows better performance than $CuCl_2$> H_2SO_4> $AlCl_3$ to remove hemicellulose from substrates. Pretreatment using $FeCl_3$ produces the maximum monomeric xylose of 94 wt % and the smallest of inhibitor product (furfural yield). Therefore, the high reducing sugar yields after enzymatic hydrolysis of substrates treated with $FeCl_3$ predictably can be produced. Hou et al. [17] have examined the effect of adding monovalent, divalent, and trivalent chloride salts to the pretreatment of *Eucalyptus* blades in steel reactors at the temperature of 160 °C, the concentration of 0.3 M, and the reaction time of 20 m. The results of this study indicate that chloride salts can accelerate the degradation of cellulose and hemicellulose from the transition, alkaline, and alkali groups, namely $FeCl_3$> $MgCl_2$> $CaCl_2$> KCl> NaCl. Pretreatment using chloride salts from transition, alkaline, and alkali groups can damage the structural density of cellulose, hemicellulose, and lignin, reduce crystallinity, reduce polymerization of cellulose, and increase porosity [17]. Research on the effect of adding $FeCl_3$ in the hydrothermal process on OPF samples has not been studied so far. Therefore, it is necessary to study the impact of adding $FeCl_3$ on the hydrothermal pretreatment process of OPF biomass to enhance reducing sugar production.

2. Methods

2.1. Preparation of OPF biomass as a feedstock

The OPF biomass was collected from an oil palm plantation owned by Citra Graha Estate, Banjarbaru, South Kalimantan, Indonesia. The OPF biomass was cut, cleaned from the debris, and dried. An air-dried OPF biomass was milled and sieved to get a 60 mesh powder. The powder of OPF biomass was stored in a plastic container at room temperature.

2.2. Hydrothermal pretreatment

The hydrothermal pretreatment of OPF samples was performed in a 35 mL internal volume. A 3 g quantity of OPF samples were added to 30 mL of iron (III) chloride ($FeCl_3$) solutions (0.075, 0.15,

0.225 M). In this study, 0.075, 0.15, and 0.225 M of $FeCl_3$ was chosen based on the results of Kamireddy et al. [16] and Hou et al. [17] studies. The hydrothermal reactor was put into the oven for the pretreatment process at 190 °C and 30 min. After 30 min, the reactor was cooled in a water bath to a temperature of 30 °C. The mixture was filtered with filter paper (Whatman No. 42). The residue was washed with distilled water until a pH range of 5.0-7.0 and dried in an oven at 105 °C for four h. The residue from hydrothermal pretreatment was then referred to as the substrate, and the liquid of filtered result was noted as filtrate. All the pretreatment experiments were conducted three times under the same conditions.

2.3. Characterization of filtrate and substrate

The pH values were measured for all solutions before and after pretreatment (fresh filtrate) using a digital pH meter (Therma Scientific Orion 3 Star). The reduced mass of the substrate was calculated gravimetrically using by the following Equation:

$$\textit{The reduced mass of the substrate (\%)} = \frac{W_{initial}-W_{Substrate}}{W_{initial}} \; x\; 100 \qquad (1)$$

where $w_{initial}$ is the mass of the OPF biomass before the pretreatment (g), and $W_{substrate}$ is the mass of the OPF biomass after pretreatment (g).

The color of the substrate was measured using a colorimeter (Precise Color Reader WR-10). The color was then characterized using a three-dimensional color parameter (CIE L*, a*, and b*). All the color measurements were conducted five times under the same conditions. The values of L^* show the lightness level; the values of a^* show the red-green color, and the values of b* show the yellow-blue color. The values of L* have a scale of 0 to 100, showing dark to bright samples. The values of a* and b * do not have a specific range. a*, which has a positive value, indicates the red color. a*, which has a negative value, indicates green color. b*, which is positive, shows yellow. b*, which is negative, indicates blue. The non-treated samples of OPF biomass were used as a control. The total color change (ΔE) of the substrate was calculated by the Equation (2) below:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \qquad (2)$$

where $\Delta L^* = L^*_{Treated} - L^*_{Non\text{-}treated}$; $\Delta a^* = a^*_{Treated} - a^*_{Non\text{-}treated}$; $\Delta b^* = b^*_{Treated} - b^*_{Non\text{-}treated}$

FTIR was used to analyze functional groups on OPF biomass before and after hydrothermal pretreatment. The samples were sent to the Organic Chemistry Laboratory of Gadjah Mada University to be characterized and measured at wavenumbers of 4000-400 cm^{-1}. The FTIR absorption data were also used to see the changes in the structure of OPF biomass like TCI (Total Crystallinity Index), LOI (Lateral Order Index), and HBI (Hydrogen Bond Intensity) parameters. The FTIR absorption data (% T) needs to be converted to absorbance (A = 2 - LOG (% T)) in order to determine these parameters. The TCI parameter was measured by comparing the absorbance value at wavenumber 1373 and 2900 cm^{-1} (A_{1373} / A_{2900}) from the FTIR spectrum. The ratio of absorbance values at peak 1427 and 894 cm^{-1} is the parameter for the LOI value (A_{1427} / A_{894}) [18, 19]. The HBI parameters were determined by the wavenumber ratio at the peak of 3400 and 1320 cm^{-1} (A_{3400} / $_{1320}$) [20].

2.4. Enzymatic hydrolysis of substrates

A 200 mg quantity of washed-pretreated solid substrate and 5 mL quantity of an acetic buffer (0.05 M and pH 5) were added into a test tube and sterilized in an autoclave at 121 °C for 15 min. A 50 mg quantity of cellulose enzymes and 5 mL quantity of a sterile acetic buffer then were added. All enzymatic hydrolysis process was carried out in a water bath shaker incubator (Lab Companion BS-11) at 50 °C and 150 rpm for 48 h. The reaction tubes were put into boiled water at 100 °C for 5 min to stop the hydrolysis reaction. Hydrolyzed samples were centrifuged for 10 min at 2000 rpm to separate

the solid residue and hydrolyzed liquid. The contents of reducing sugars in the hydrolyzed liquid were determined by the DNS method using UV-visible spectroscopy (Genesys 10 UV).

2.5 Reducing sugars analysis using DNS methods

Reducing sugars analysis using DNS methods. The DNS reagent is a solution formed by the following compounds: 1 g of 3,5-Dinitrosalicylic acid, 0.2 g of phenol, 0.05 g of Na_2SO_3, 20 g of Rochelle salt. These were added into 50 mL of NaOH 2% (w/v) and stirred to a homogeneous solution. The solution was then transferred into the volumetric flask (100 mL) and completed with distilled water to the etched line. To measure the absorbance of standard glucose of different concentrations (0.1, 0.125, 0.15, 0.175, 0.2 g/L), 1.5 mL of a standard glucose solution was mixed with 3 mL DNS reagent, positioned in a boiling water bath for 5 min, cooled to room temperature. One of standard glucose solutions (0.15 g/L glucose) was taken for wavelength from 500 to 600 nm to get the optimal operating wavelength, which gives the highest sensitivity. The maximum absorbance wavelength corresponded to 548 nm. It was used to measure the absorbance of standard glucose and hydrolyzed liquid samples. The absorbance values of standard glucose were then plotted to obtain a standard curve. The concentration of reducing sugars in hydrolyzed liquid samples was obtained by converting its absorbance into a standard curve graph equivalent.

2.6 Statistical analysis

Experimental data were statistically analyzed by analysis of variance (ANOVA), and samples showing significant differences ($P < 5\%$) were analyzed using the Tukey test. All analyses were performed using IBM SPSS Statistics.

3. Results and discussions

3.1. Characteristic analysis

3.1.1. The effect of $FeCl_3$ concentration on the pH of the filtrate. The pH values were measured for all solutions before (deionized water mixed with $FeCl_3$) and after pretreatment (noted as filtrate). The pH values of all pretreatment solutions with various concentration (0.1, 0.2, 0.3 M) were found acidic (Ph < 2). The pH values of all filtrate decreased, as shown in Figure 1. The pH value of the solution without the addition of the $FeCl_3$ concentration of 5.725 decreased to 5.596. The pH value of the solution with $FeCl_3$ 0.075 M concentration of 1.888 decreased to 1.818. Then, the pH values of the solution with $FeCl_3$ concentration of 0.15 M and 0.225 M of 1.636 and 1.398 decreased to 1.264 and 0.938, respectively. These results were in agreement with the study by Kamireddy et al. [16]. The decreased pH values of the solution after the pretreatment process indicates the degradation of hemicellulose to form acetic acid in the filtrate. Acetic acid is what causes the pH of the solution after the pretreatment process becomes acidic. The formation of acetic acid is mainly caused by two functional groups esters and acetyl in lignocellulose biomass. The acetyl group connects the monosaccharide in the polymer, and the ester group is the acetyl substituent in the hemicellulose component. During the pretreatment process, the acetyl bond in the hemicellulose breaks down to form acetic acid in the filtrate [16].

3.1.2. The effect of $FeCl_3$ concentration on the reduced mass of the substrate. The effect of $FeCl_3$ concentration on hydrothermal pretreatment of OPF on the reduced mass of substrate was analyzed gravimetrically. The presence of $FeCl_3$ concentration can accelerate the percentage of reduced mass of substrate, indicating the decomposition of OPF lignocellulosic structures. The rate of reduced substrate mass increased significantly with the increase of $FeCl_3$ concentration, as shown in Figure 2. The percentage of reduced mass of substrate increased from 15.232% to 20.67% at $FeCl_3$ 0.075 M. The rate of reduced mass of substrate then increased to 32% and 51.481% at $FeCl_3$ 0.15 M and 0.225 M. It is assumed that hemicellulose was degraded on the filtrate and lignin was degraded or dissolved partially. Most of the inorganic salt on the pretreatment process can catalyze the hydrolysis reaction of lignocellulose in a relevant report [14, 17, 21].

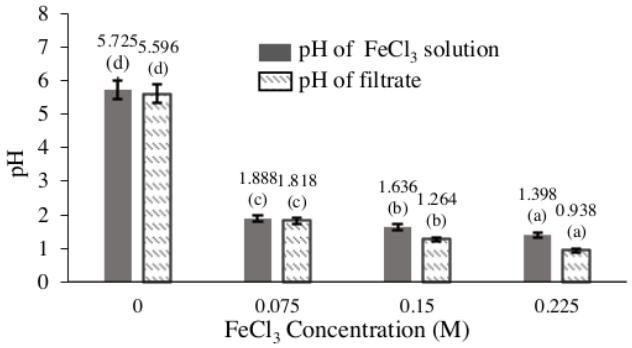

Figure 1. The effect of $FeCl_3$ concentration (M) on the pH of the filtrate.

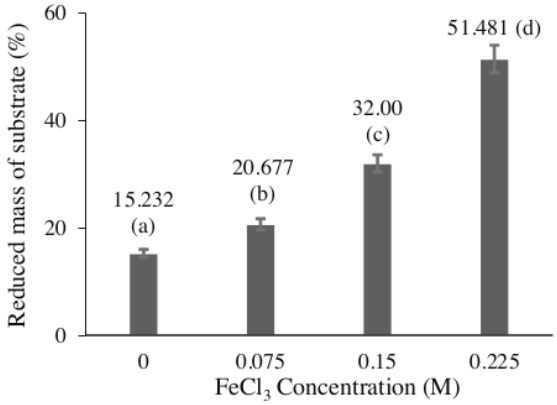

Figure 2. The effect of $FeCl_3$ concentration (M) on a reduced mass of substrate.

The Fe^{3+} ions in aqueous solution act as Lewis acids, producing complex cations $[Fe(H_2O)_6]^{3+}$ where they undergo hydrolysis reaction with water molecules producing H_3O^+ ions. The hydrolysis reactions are shown in Equation (3) and (4). The positive charge on the Fe^{3+} ion draws electron density from the O-H bond in the water. This electron density increases the bond's polarity making it easier to break. An aqueous proton is released, producing an acidic solution and giving the lower pKa value (acid dissociation constant) when the O-H bond breaks. The lowest pKa value of $FeCl_3$ in water solution (2.46) indicates the strong acidic character [16]. However, the firm acidity of the solution to the pretreatment process can aid the hydrolysis reaction of OPF biomass.

$$[Fe(H_2O)_6]^{3+} + H_2O \rightleftharpoons [Fe(H_2O)_5OH]^{2+} + H_3O^+ \quad (3)$$

$$[Fe(H_2O)_5OH]^{2+} + H_2O \rightleftharpoons [Fe(H_2O)_4(OH)_2]^{+} + H_3O^+ \quad (4)$$

When the concentration of $FeCl_3$ was enlarged from 0.075 M to 0.225 M, the reduced mass of substrates increased significantly. When the acidity of the solution reaches to 1.398, Fe^{3+} ions provide more empty orbitals. The unoccupied orbitals of Fe^{3+} ions producing the complexes of xylan-Fe^{3+}. The bond angle and the bond length between the C atom and O atom in the pyran ring become more extensive and lead the ring-opening reaction between C1 and C2 routing the degradation of the glycosidic bond to oligosaccharide or monosaccharide in the filtrate.

3.1.3. The effect of $FeCl_3$ concentration on the color of the substrate and filtrate. The hydrothermal pretreatment of OPF biomass using the addition of $FeCl_3$ concentration can cause changes in the substrate color and filtrate color. The color variations of substrate and filtrate during hydrothermal pretreatment were caused by a series of chemical reactions between the lignocellulosic constituents of OPF biomass under high temperature and acidic conditions. The color change in substrate and filtrate can be seen visually in Figure 3, which shows the substrate and filtrate with $FeCl_3$ concentration of 0.225 M during hydrothermal was darkest. Quantitative data with the colorimeter test of the color change of substrates and filtrates have also occurred.

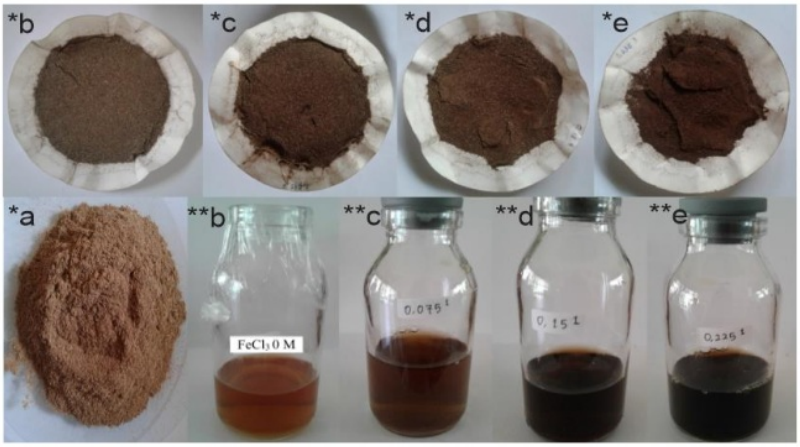

Figure 3. The color of the substrate (*) and filtrate (**). (a) untreated sample of OPF biomass (b) without the addition of $FeCl_3$; (c) $FeCl_3$ concentration of 0.075 M; (d) $FeCl_3$ concentration of 0.150 M; (e) $FeCl_3$ concentration of 0.225 M.

Figure 4. shows the mean values of the color parameters of L*, a*, and b* of the substrate. The color characteristics of the substrate are the reflection of the pretreatment conditions. The lightness (L*) of the substrate decreased by up to a maximum of about 19.268% with increasing $FeCl_3$ concentration. The value of a* and b* substrate increased significantly with increasing $FeCl_3$ concentration. The total color difference (ΔE*) is a good indicator of color deviation between pretreated and non-treated samples. The highest absolute color difference was observed at 0.225 M. It implied that the acidic condition in the pretreatment process could change the chemical structure of OPF biomass. The most severe and enormous amounts of polysaccharides with low molecular weights were created due to the degradation of celluloses and hemicelluloses. Cellulose does not change more than hemicellulose because cellulose is more stable. Hemicellulose is more sensitive to temperature and more accessible to degrade than cellulose, especially in a high humidity environment. Lignin and extractives can also contribute to color changes. Chromophoric groups can be produced within the lignin molecules or extractive molecules. The concentrations of the carboxylates and hydroxy benzenes can increase, and the color of OPF biomass will darken. Therefore, the darkest shade was observed [22].

Figure 5. shows the data of filtrate color after the sample was treated with various of $FeCl_3$ concentration. It can be seen that a* dan b* value of filtrate was a fluctuant trend, while the other both of L* and ΔE saw a decrease and an upward trend. The filtrate color became darker with increasing $FeCl_3$ concentration during the pretreatment process because of the dissolved lignocellulosic components in the filtrate, such as lignin.

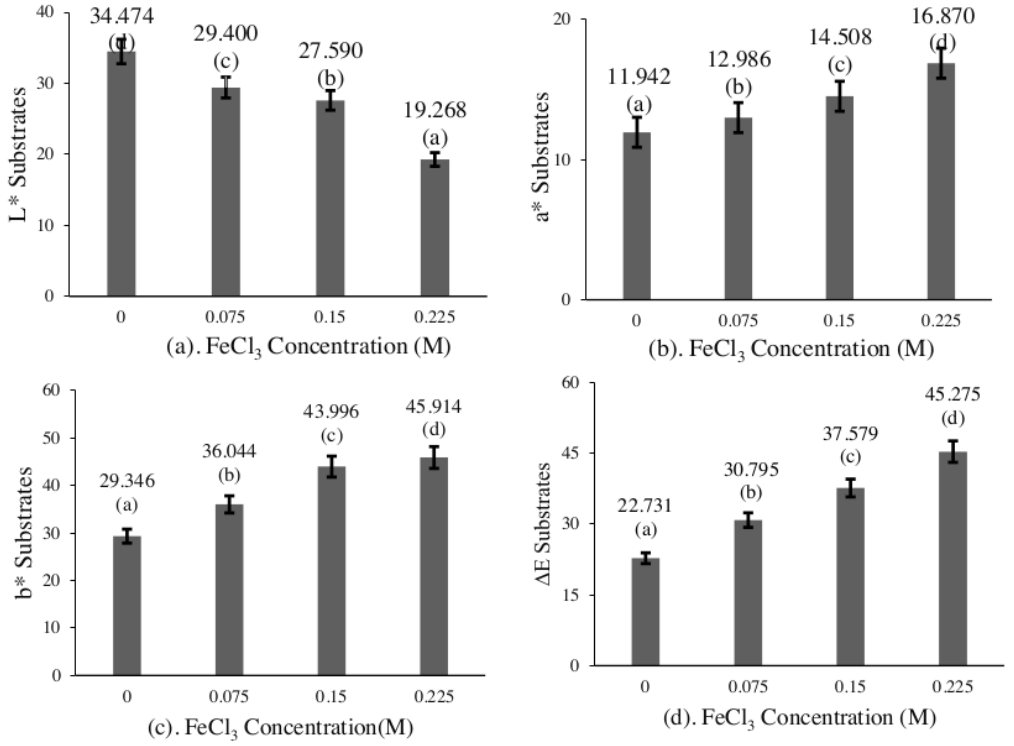

Figure 4. Substrate color changes after hydrothermal pretreatment at different concentrations. Variation of CIE LAB parameters: (a). L*, (b). a*, (c). b*, and (d) ΔE.

3.1.4. FTIR analysis. The effects of $FeCl_3$ concentration on the chemical composition of OPF substrates were evaluated using FTIR spectroscopy, as shown in Figure 6. The peak at 3455–3230 cm^{-1} indicates the presence of the hydroxyl group [23]. According to Shan[29]t al. [21], it represents the intramolecular or intermolecular hydrogen bonds in cellulose. The peak at 894 cm^{-1} is a characteristic peak of a β-glycosidic bond between cellulose and hemicellulose [21]. This peak of OPF biomass (untreated samples) did not appear in the spectra. The peak then appearred in the substrate without[21] addition of $FeCl_3$ concentration in 894.27 and 894.97 cm^{-1} for substrates (0.075, 0.15, 0.225 M). The intensity of each peak decreased with increasing concentration of $FeCl_3$, indicating that the increase of $FeCl_3$ concentration on hydrothermal pretreatment could catalyze the deconstruction of lignocellulosic structure so that amorphous cellulose appearred in the substrate. The peak shift at 1735.93 cm^{-1} to 1728.22 cm^{-1} occurr[28] when the $FeCl_3$ concentration of 0.225 M indicating that it could damage the ester bond between lignin and polysaccharide. The peak at 1249.87 cm^{-1} decreased the wave number when the $FeCl_3$ concentrations of 0.075, 0.15, and 0.225 M were used. The decrease in wavenumber and peak intensity indicates that $FeCl_3$ can break ether bonds between lignin and polysaccharides [21]. Peak intensity at wave number 1510-1515 cm^{-1}, which decreases with increasing concentration of $FeCl_3$, shows a change in lignin composition caused by the release and redeposition of lignin to form droplets on the surface of the substrate [23-25].

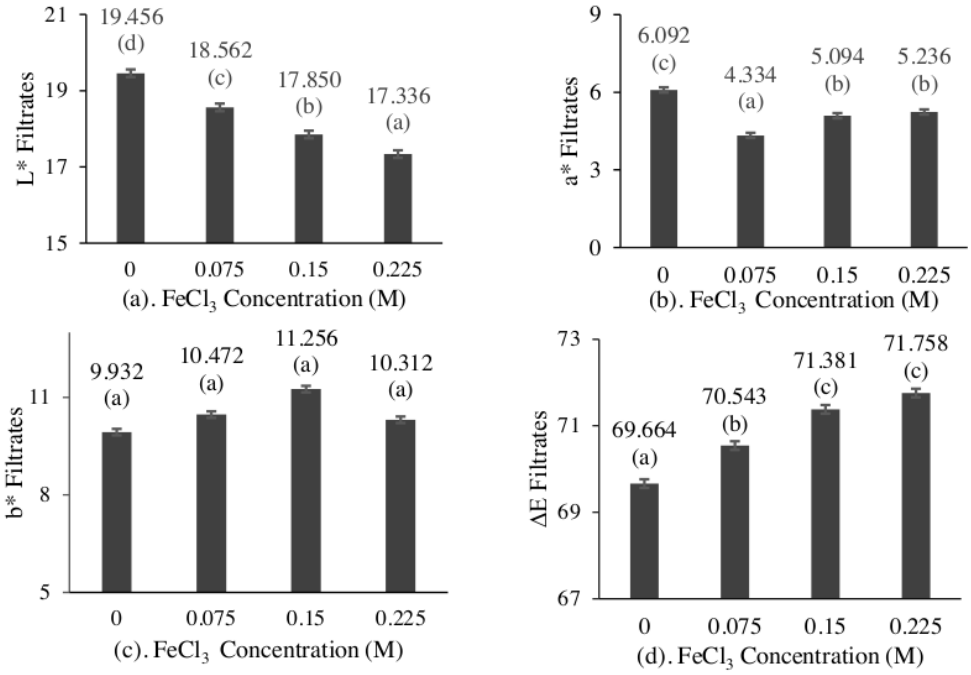

Figure 5. Filtrate color changes after hydrothermal pretreatment at different concentrations. Variation of CIE LAB parameters: (a). L*, (b). a*, (c). b*, and (d) ΔE.

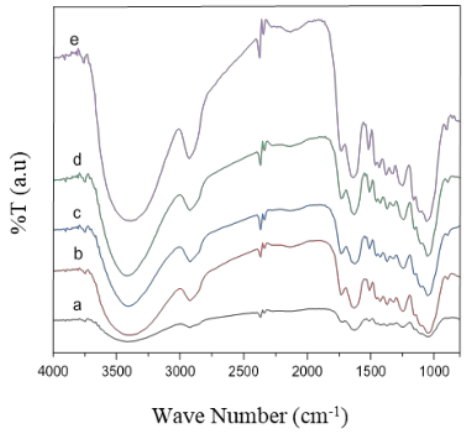

Figure 6. FTIR Spectra of OPF Biomass before and after pretreatment process. (a) un-treated samples; (b) without the addition of $FeCl_3$; (c) $FeCl_3$ concentration of 0.075 M; (d) $FeCl_3$ concentration of 0.150 M; (e) $FeCl_3$ concentration of 0.225 M.

The data of FTIR spectra were then used to calculate the values of TCI, LOI, and HBI substrate, as shown in Table 1. The value of TCI and LOI was used for the determination of cellulose crystallinity. Both of TCI and LOI values decreased drastically when 0.225 M of $FeCl_3$ was used. The TCI value of substrate treated with 0; 0.075; 0.15 M $FeCl_3$ was slightly divergent, but the decrease of TCI value of substrate treated with 0.225 M $FeCl_3$ occurred drastically. The LOI values of substrates treated without the addition of $FeCl_3$ and with the highest $FeCl_3$ (0.225 M) plunged, from 1.193 to 0.675. It indicates

that pretreatment using $FeCl_3$ of 0.225 M can change cellulosic structures from cellulose of type II (an ordered structure) to cellulose of type II, which is not an organized structure (more amorphous form). The HBI values of each substrate were slightly different, but they increased when they were used by 0.225 M of $FeCl_3$. The decreased LOI value indicates that lignocellulose after pretreatment has increased in cellulose surfaces' accessibility and will theoretically enhance the efficiency of enzymatic hydrolysis [26]. The rise of HBI values is due to the release of hemicellulose and lignin polymer, which protect cellulose structure. Consequently, intra and intermolecular hydrogen bonds of cellulose become more exposed in the substrates.

Table 1. The values of TCI, LOI, and HBI based on FTIR spectra.

$FeCl_3$ concentration (M)	TCI	LOI	HBI
Without addition of $FeCl_3$ Concentration	1.036	1.193	1.453
0.075	0.998	1.145	1.403
0.150	0.994	1.086	1.451
0.225	0.559	0.675	1.510

3.2 Reducing sugars analysis

The results of reducing sugar concentration on hydrolyzed liquid samples are presented in Figure 7. The highest reducing sugar concentration of 3.8 g/L was revealed on sample 4, which was pretreated with 0.225 M as compared with 0, 0.075, 0.15 M of $FeCl_3$ concentration for other samples. It is assumed that the pretreated-OPF sample with 0.225 M of $FeCl_3$ concentration has an opened structure based on TCI, LOI, HBI value of substrates. An opened structure of substrates increases the accessibility of cellulosic enzymes to hydrolyze polysaccharide to monosaccharide. Based on the results, the presence of $FeCl_3$ on hydrothermal pretreatment can catalyze the hydrolysis reaction of OPF samples and enhance the reducing sugar production of enzymatic hydrolysis.

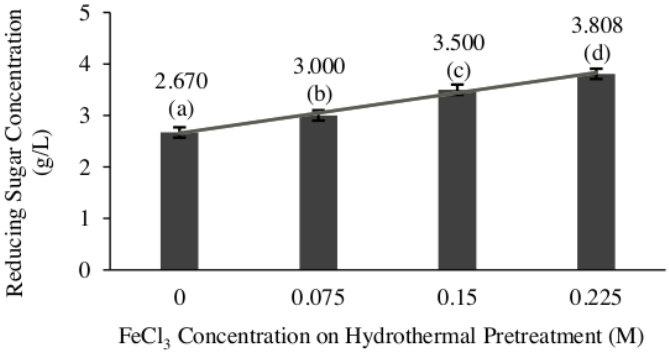

Figure 7. The correlation between reducing sugar concentration and $FeCl_3$ concentration.

3.3 Correlation analysis

Figure 8 shows the correlation analysis between the substrate characteristics and the amount of reducing sugar concentration. The p-value of the test (a) is 0.043, which is less than the significance level alpha = 0.05. While the p-value of the test b (TCI = 0.217, LOI = 0.151, and HBI = 0.344) is higher than the significance level alpha = 0.05. We can conclude that the reduced mass of substrate and reducing sugar concentration are significantly correlated with a correlation coefficient of 0.957 and a p-value of 0.043. The alteration of TCI, LOI, and HBI values does have an insignificant correlation.

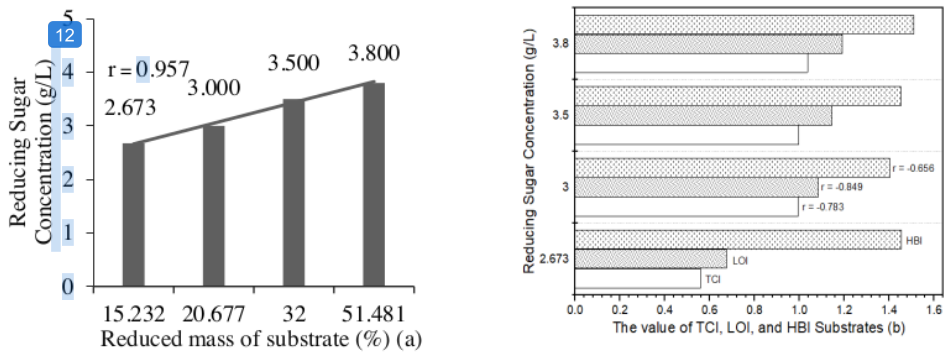

Figure 8. The correlation between the reduced mass of substrate (a), the value of TCI, LOI, and HBI substrate (b) and the amount of reducing sugar.

4. Conclusion

The alteration of substrates and filtrates confirmed the changes in OPF lignocellulose. $FeCl_3$ used on the hydrothermal pretreatment process could catalyze the hydrolysis reaction of OPF lignocellulose, giving the accessibility of enzymes to enhance reducing sugar concentration. Moreover, the substrate treated with the highest $FeCl_3$ (0.225 M) produces the most elevated reducing sugar after enzymatic hydrolysis. Based on our data, the increase of $FeCl_3$ concentration will increase reducing sugar production.

5. References

[1] Nadia A, Fauziah A, Mayori E and Sunardi 2018 Potential of lignocellulosic oil palm waste in South Kalimantan for bioethanol and xylitol prodcution (in Indonesian) *Quantum: Jurnal Inovasi Pendidikan Sains* **8**(2): 41-51

[2] Lai L W, Ibrahim M, Rahim N M, Hashim E F, YA'COB M Z, Idris A and Akhtar J 2016 Study on composition, structural and property changes of oil palm frond biomass under different pretreatments *Cellul. Chem. Technol* **50**: 951-959

[3] Sunardi, Febriani N M and Junaidi A B 2017 Preparation of carboxymethyl cellulose produced from Purun Tikus (Eleocharis Dulcis) *AIP Conference Proceedings* **1868** 020008

[4] Nurwahdah N, Naini A A, Nadia A, Lestari R Y and Sunardi P D S 2018 Lignocellulose pretreatment of rice straw using deep eutectic solvent to increase second-generation bioethanol production (in Indonesian) *J. Ris. Ind.Has Hutan* **10**(1): 43-54

[5] Hermiati E, Mangunwidjaja D, Sunarti T C, Suparno O and Prasetya B 2017 Utilization of lignocellulosic biomass from sugarcane bagasse for bioethanol production (in Indonesian) *JPPP* **29**(4): 121-130

[6] Menon V and Rao M 2012 Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept *Progr Energ Combust* **38**: 522-550

[7] Fan S, Zhang P, Li F, Jin S, Wang S and Zhou S 2016 A review of lignocellulose change during hydrothermal pretreatment for bioenergy production *Curr. Org. Chem.* **20**: 2799-2809

[8] Petersen M Ø, Larsen J and Thomsen M H 2009 Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals *Biomass and Bioenerg* **33**: 834-840

[9] Sun T-S, Wang K, Yang G, Yang H-Y and Xu F 2014 Hydrothermal treatment and enzymatic saccharification of corncobs *BioResources* **9**(2): 3000-3013

[10] Fang C, Schmidt J E, Cybulska I, Brudecki G P, Frankær C G and Thomsen M H 2015 Hydrothermal pretreatment of date palm (*Phoenix dactylifera* L.) leaflets and rachis to enhance enzymatic digestibility and bioethanol potential *Biomed Res Int* **2015** 216454

[11] Nitsos C K, Choli-Papadopoulou T, Matis K A and Triantafyllidis K S 2016 Optimization of hydrothermal pretreatment of hardwood and softwood lignocellulosic residues for selective

hemicellulose recovery and improved cellulose enzymatic hydrolysis *ACS Sustain Chem Eng* **4**: 4529-4544

[12] Nadia A, Sunardi S and Rodiansono R 2018 Hydrothermal pretreatment of oil palm fronds for increasing enzymatic saccharification *AIP Conference Proceedings* **2021** 030002

[13] Moodley P and Gueguim Kana E B 2017 Development of a steam or microwave-assisted sequential salt-alkali pretreatment for lignocellulosic waste: effect on delignification and enzymatic hydrolysis *Energ Convers Manage* **148**: 801-808

[14] Liu L, Sun J, Cai C, Wang S, Pei H and Zhang J 2009 Corn stover pretreatment by inorganic salts and its effects on hemicellulose and cellulose degradation *Bioresour. Technol* **100**: 5865-5871

[15] Li S and Song X 2018 Study on the preparation and production factors of a direct lignocellulose biomass fuel cell *Journal Electroanal. Chem.* **810**: 55-61

[16] Kamireddy S R, Li J, Tucker M, Degenstein J and Ji Y 2013 Effects and mechanism of metal chloride salts on pretreatment and enzymatic digestibility of corn stover *Ind. Eng. Chem* **52**: 1775-1782

[17] Huo D, Danni X, Yang Q, Liu Q, Hou Q and Tao Z 2018 Improving the efficiency of biomass pretreatment and enzymatic saccharification process by metal chlorides *BioResources* **13**(1): 1909-1916

[18] Nelson M L and O'Connor R T 1964 Relation of certain infrared bands to cellulose crystallinity and crystal latticed type. Part I. spectra of lattice types I, II, III and of amorphous cellulose *J. Appl.Polym. Sci* **8**: 1311-1324

[19] O'Connor R T, DuPré E F and Mitcham D 1958 Applications of infrared absorption spectroscopy to investigations of cotton and modified cottons *Text. Res. J.* **28**: 382-392

[20] Auxenfans T, Crônier D, Chabbert B and Paës G 2017 Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment *Biotechnol Biofuels* **10**: 36-36

[21] Shang Y, Chen M, Zhao Q, Su R, Huang R, Qi W and He Z 2017 Enhanced enzymatic hydrolysis of lignocellulose by ethanol-assisted fecl 3 pretreatment *Chem. Eng. Trans* **61**: 781-786

[22] Zhang Y and Cai L 2005 Effects of steam explosion on wood appearance and structure of sub-alpine fir *Wood Sci Technol* **40**: 427-436

[23] Sunardi S, Istikowati W T, Ishiguri F and Yokota S 2018 Ftir spectroscopy and color change of wood for assessment and monitoring of softwood degradation by white-rot fungus porodaedalea pini *AIP Conference Proceedings* **2026** 020024

[24] Li J, Henriksson G and Gellerstedt G 2007 Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion *Bioresour. Technol.* **98**: 3061-3068

[25] Romero I, Ruiz E and Castro E 2016 Pretreatment with metal salts *Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery* ed S I Mussatto (Elsevier) pp 209-227

[26] Spiridon I, Teaca C A and Bodîrlău R 2011 Structural changes evidenced by ftir spectroscopy in cellulose materials after pre-treatment with ionic liquid and enzymatic hydrolysis *BioResources* **6**: 400-413

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