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

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Abstract. The effects of FeCl₃ 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 FeCl₃ 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 FeCl₃ added (2.673 g/L). Overall, our study concludes that the hydrothermal pretreatment assisted by FeCl₃ 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 be 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 [15].

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 FeCl₃ 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:

$$\text{The reduced mass of the substrate (\%)} = \frac{W_{\text{initial}} - W_{\text{Substrate}}}{W_{\text{initial}}} \times 100 \quad (1)$$

where w_{initial} is the mass of the OPF biomass before the pretreatment (g), and $W_{\text{Substrate}}$ 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:

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

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

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 ($A = 2 - \text{LOG} (\% T)$) 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} / A_{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 Na₂SO₃, 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 on 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 FeCl₃ concentration on the pH of the filtrate. The pH values were measured for all solutions before (deionized water mixed with FeCl₃) 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 ($\text{pH} < 2$). The pH values of all filtrate decreased, as shown in Figure 1. The pH value of the solution without the addition of the FeCl₃ concentration of 5.725 decreased to 5.596. The pH value of the solution with FeCl₃ 0.075 M concentration of 1.888 decreased to 1.818. Then, the pH values of the solution with FeCl₃ 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 FeCl₃ concentration on the reduced mass of the substrate. The effect of FeCl₃ concentration on hydrothermal pretreatment of OPF on the reduced mass of substrate was analyzed gravimetrically. The presence of FeCl₃ 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 FeCl₃ concentration, as shown in Figure 2. The percentage of reduced mass of substrate increased from 15.232% to 20.67% at FeCl₃ 0.075 M. The rate of reduced mass of substrate then increased to 32% and 51.481% at FeCl₃ 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].

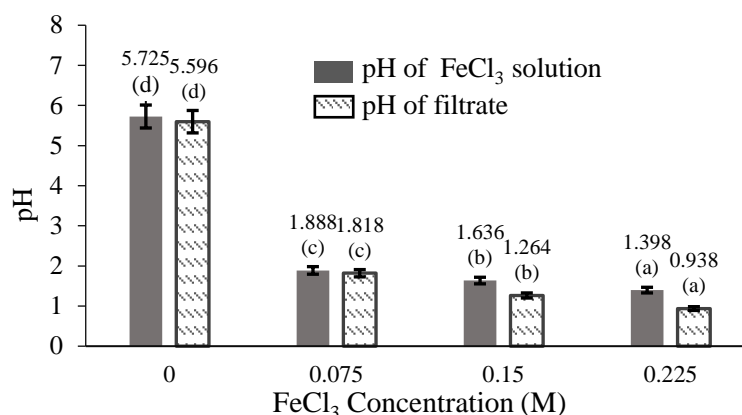


Figure 1. The effect of FeCl₃ concentration (M) on the pH of the filtrate.

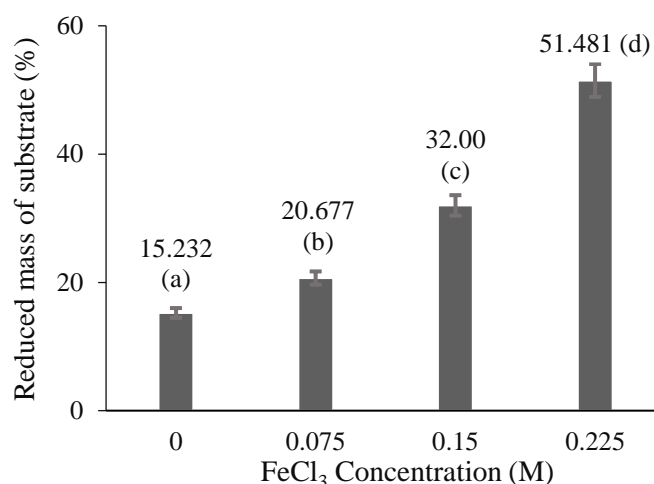
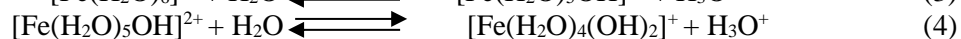
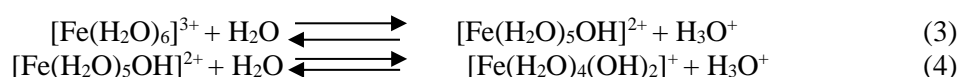


Figure 2. The effect of FeCl₃ concentration (M) on a reduced mass of substrate.

The Fe³⁺ ions in aqueous solution act as Lewis acids, producing complex cations [Fe (H₂O)₆]³⁺ where they undergo hydrolysis reaction with water molecules producing H₃O⁺ ions. The hydrolysis reactions are shown in Equation (3) and (4). The positive charge on the Fe³⁺ 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 pK_a value (acid dissociation constant) when the O-H bond breaks. The lowest pK_a value of FeCl₃ 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.



When the concentration of FeCl₃ 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, Fe³⁺ ions provide more empty orbitals. The unoccupied orbitals of Fe³⁺ ions producing the complexes of xylan-Fe³⁺. 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 FeCl₃ concentration on the color of the substrate and filtrate. The hydrothermal pretreatment of OPF biomass using the addition of FeCl₃ 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 FeCl₃ 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.

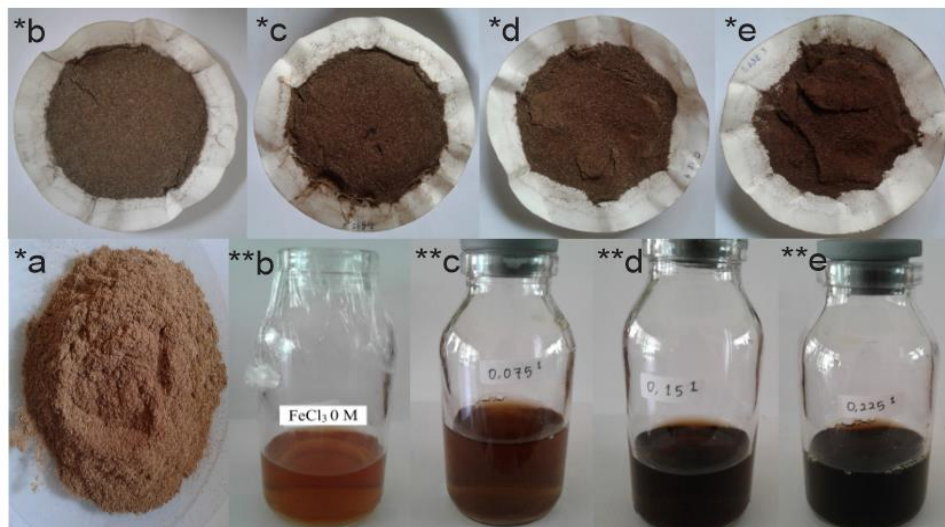


Figure 3. The color of the substrate (*) and filtrate (**). (a) untreated sample of OPF biomass (b) without the addition of FeCl₃; (c) FeCl₃ concentration of 0.075 M; (d) FeCl₃ concentration of 0.150 M; (e) FeCl₃ concentration of 0.225 M.

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 FeCl₃ concentration. The value of a* and b* substrate increased significantly with increasing FeCl₃ 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 FeCl₃ 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 FeCl₃ concentration during the pretreatment process because of the dissolved lignocellulosic components in the filtrate, such as lignin.

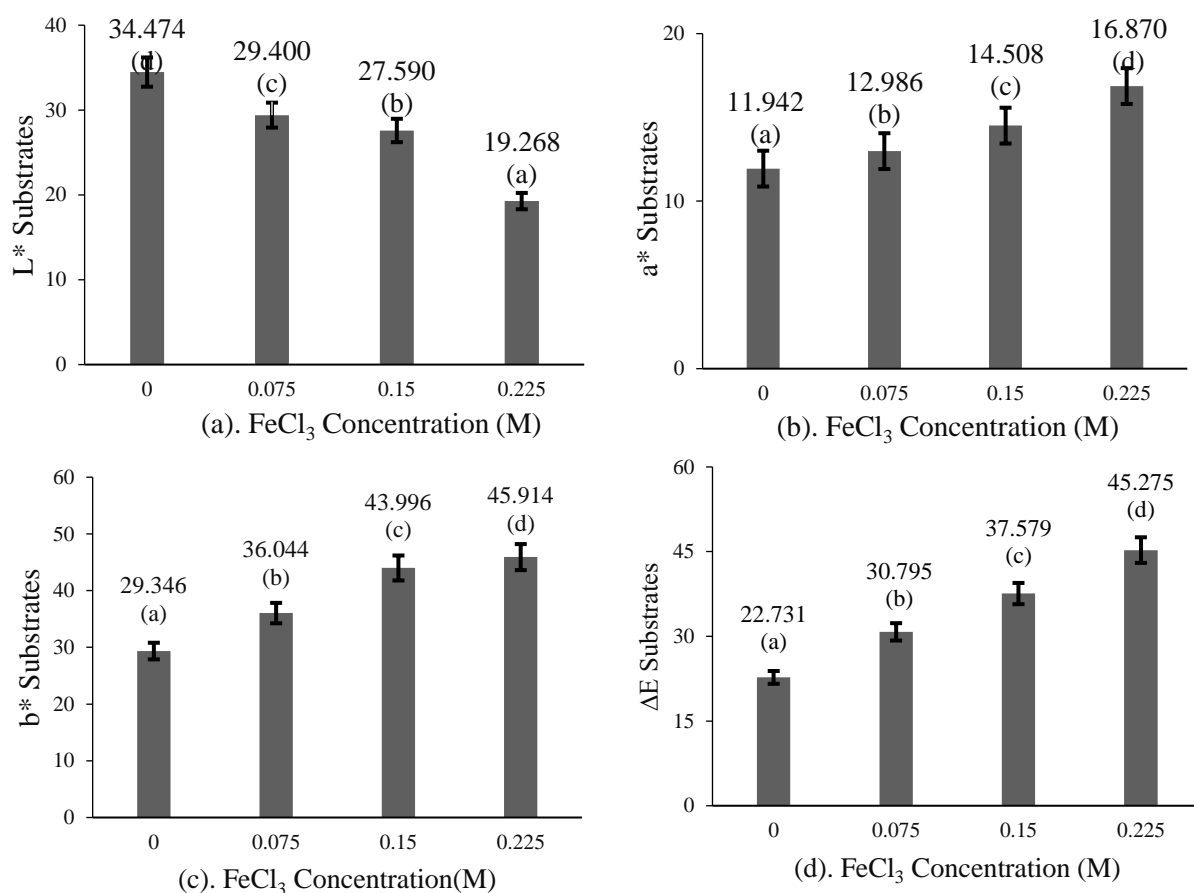


Figure 4. Substrate color changes after hydrothermal pretreatment at different concentrations. Variation of CIE LAB parameters: (a). L*, (b). a*, (c). b*, and (d) ΔE.

3.1.4. FTIR analysis. The effects of FeCl₃ 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 Shang et 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 appeared in the substrate without the addition of FeCl₃ 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 FeCl₃, indicating that the increase of FeCl₃ concentration on hydrothermal pretreatment could catalyze the deconstruction of lignocellulosic structure so that amorphous cellulose appeared in the substrate. The peak shift at 1735.93 cm⁻¹ to 1728.22 cm⁻¹ occurred when the FeCl₃ 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 FeCl₃ concentrations of 0.075, 0.15, and 0.225 M were used. The decrease in wavenumber and peak intensity indicates that FeCl₃ can break ether bonds between lignin and polysaccharides [21]. Peak intensity at wave number 1510–1515 cm⁻¹, which decreases with increasing concentration of FeCl₃, 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].

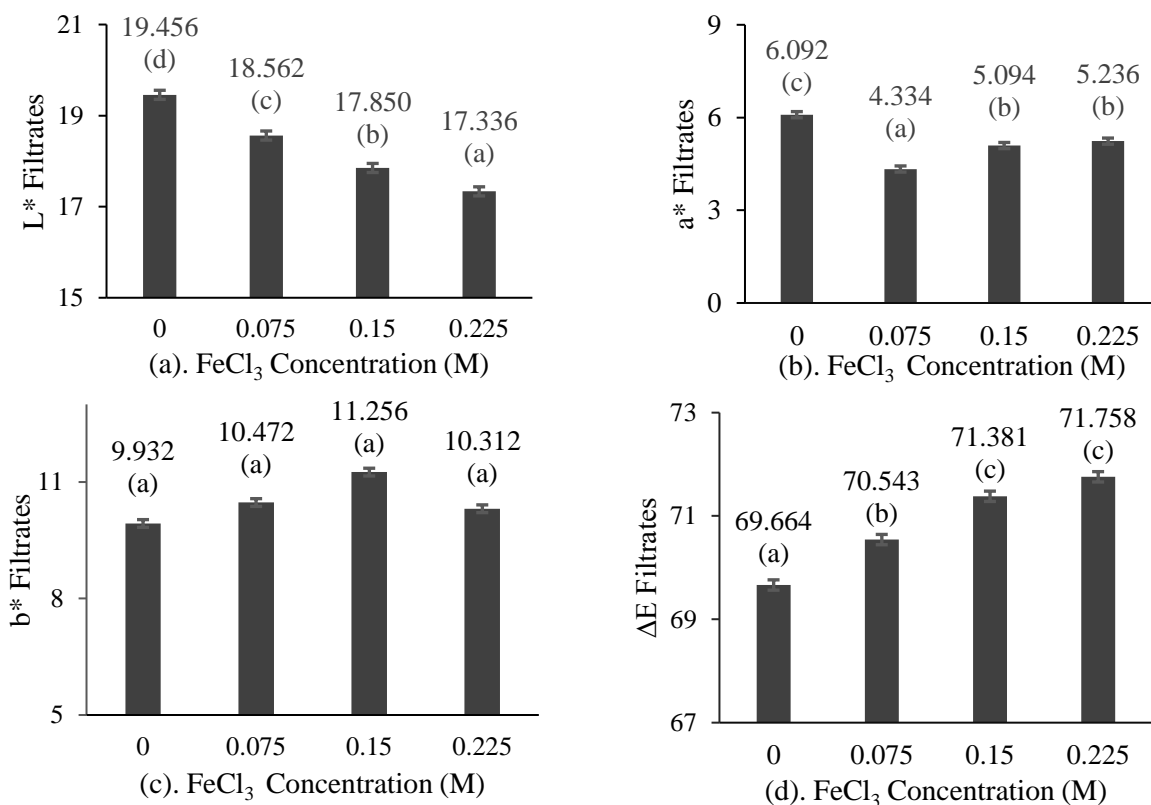


Figure 5. Filtrate color changes after hydrothermal pretreatment at different concentrations. Variation of CIE LAB parameters: (a). L*, (b). a*, (c). b*, and (d) ΔE.

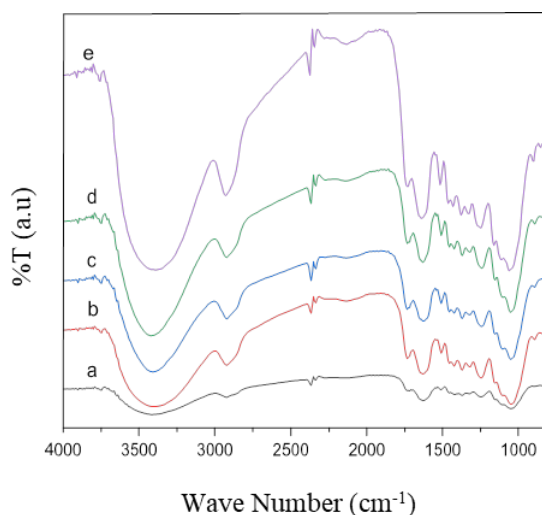


Figure 6. FTIR Spectra of OPF Biomass before and after pretreatment process. (a) un-treated samples; (b) without the addition of FeCl₃; (c) FeCl₃ concentration of 0.075 M; (d) FeCl₃ concentration of 0.150 M; (e) FeCl₃ concentration of 0.225 M.

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 FeCl₃ was used. The TCI value of substrate treated with 0; 0.075; 0.15 M FeCl₃ was slightly divergent, but the decrease of TCI value of substrate treated with 0.225 M FeCl₃ occurred drastically. The LOI values of substrates treated without the addition of FeCl₃ and with the highest FeCl₃ (0.225 M) plunged, from 1.193 to 0.675. It indicates

that pretreatment using FeCl₃ 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 FeCl₃. 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 FeCl₃ concentration for other samples. It is assumed that the pretreated-OPF sample with 0.225 M of FeCl₃ 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 FeCl₃ on hydrothermal pretreatment can catalyze the hydrolysis reaction of OPF samples and enhance the reducing sugar production of enzymatic hydrolysis.

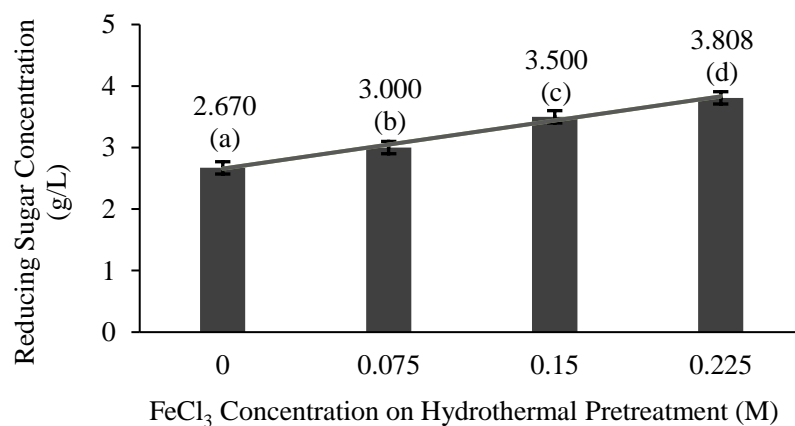


Figure 7. The correlation between reducing sugar concentration and FeCl₃ concentration.

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.

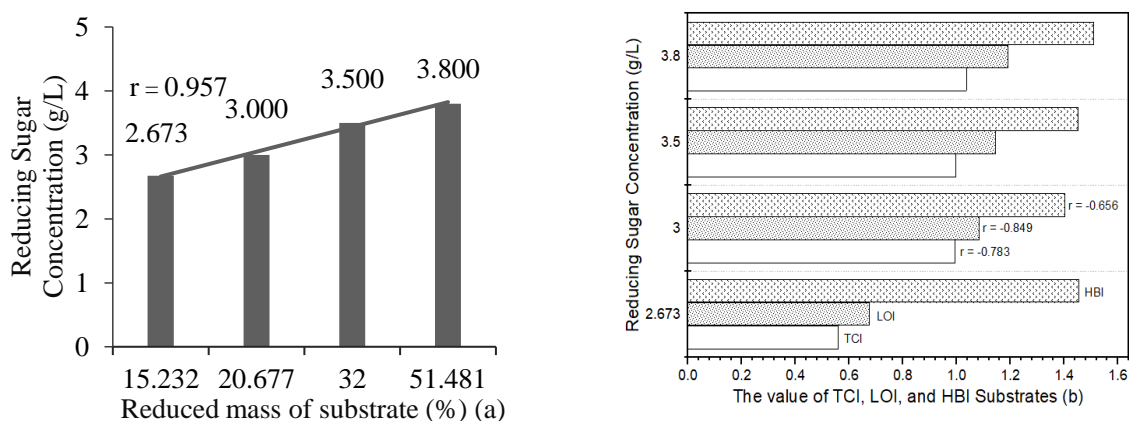


Figure 8. The correlation between the reduced mass of substrate (a), the value of TCI, LOI, and HBI substrate (b) and the amount of reducing sugar.

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.

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