Lignin black liquor degradation on oil palm empty fruit bunches using ilmenite (FeO.TiO$_2$) and its activity as antibacterial

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Abstract. Lignin from oil palm empty fruit bunches (OPEFB) has been degraded using ilmenite mineral (FeO.TiO$_2$). The FeO.TiO$_2$ was examined for antibacterial activity against $E.\ coli$, $S.\ aureus$, $S.\ typhi$, and $X.\ oryzae$. It was extracted from iron sands by a magnetic separator and pre-oxidized at 800°C for 5 hours. Meanwhile, OPEFB was prepared with a pretreatment process using 10% NaOH solution to damage ester bonds between lignin, hemicellulose, and cellulose. Furthermore, the lignin was degraded by using FeO.TiO$_2$ catalyst with the study of catalyst mass, degradation time, and lignin concentration variation. Based on XRF and XRD data indicated that the pre-oxidation result of iron sand was contained Fe$_2$O$_3$ (hematite) and FeO.TiO$_2$ minerals with the highest dominant of Fe content in the sample. The result of lignin photodegradation using FeO.TiO$_2$ catalyst showed that the lignin-derived compound obtained was Coniveryl Alcohol (C$_{10}$H$_{12}$O$_3$). Antibacterial activity test against 4 bacterial samples i.e $E.\ coli$, $S.\ aureus$, $S.\ typhi$, and $X.\ oryzae$ showed that each test has good activity in inhibiting bacteria with a range of inhibiting diameter zone between ±20 nm. Based on this study provides that lignin-derived compounds from OPEFB can be used as a natural antibacterial.

1. Introduction

The oil palm processing is a concern for a few decades causes it is required human life as an oil for household, industrial, and biofuel (biodiesel and bioethanol) [1,2]. Southeast Asia is the largest producer and exporter of palm oil in the world. In 2016s Indonesia is a country which produces 33 million tons palm oil with a total area of 11.9 million hectares, it is categorized the largest producer and exporter competing with Malaysia [3,4]. In addition, the increased palm oil production also increases the waste of palm oil as palm empty fruit bunches (OPEFB). The processing of 1.0 ton fresh fruit bunches (FFB) will be produced of 22-23% OPEFB waste, it is the same with 220-230 kg of OPEFB It is very abundant so that needs attention to reuse or proper processing [5,6].
The OPEFB waste contained natural chemical compounds such as cellulose, hemicellulose, and lignin [7]. The cellulose as the largest fraction in OPEFB, usually is used for adsorbent processed material, pulp, organic fertilizer, cellulose acetate, and paper industry [8]. Many researchers has studied by converting of glucose using cellulase enzyme in fermentation method to produce the bioethanol [9]. In general, OPEFB waste treatment through pre-treatment method by using NaOH solvent to obtain a black liquor which containing the lignin [10]. This method has been widely used by previous researchers that effectively for hemicellulose separation. Waste black liquor was contained high lignin that advantages for biofuel production and bio-pesticide based on lignin degradation principle. Lignin in black liquor is a general chemical in OPEFB waste that difficult to degrade caused consisting the aromatic group which composed of propane phenyl units such as paracoumaryl alcohol, coniferyl alcohol, and sinapyl alcohol [11]. It is can be separated by photooxidation process using catalyst-assisted as though Titanium dioxide (TiO$_2$) [12-14].

The TiO$_2$ material was currently needed intensive in many countries due to high stability, inert, high optical properties, non-toxic, and eco-friendly to fabricate the engineering materials [15-20]. In addition, it can be found in the natural as ilmenite mineral (FeO.TiO$_2$) [21-24]. Indonesia has an abundance of FeO.TiO$_2$ mineral but it is not utilized optimally [25,26]. In this study, we have conducted the iron sand leaching extraction that can be used as a catalyst to degrade lignin from OPEFB. We are using FeO.TiO$_2$ catalyst due to contain TiO$_2$ and FeO have high photocatalyst under ultraviolet (UV) irradiation to produce phenolic and non-phenolic units derivatives from lignin. The phenolic derivates commonly highly active because of substituted -OH groups in benzene group an important role as antimicrobial agents.

2. Experimental Methods

2.1. Extraction of iron sand
The iron sand was obtained from Southeast Sulawesi and dried in an oven (Memmert um 400) at 105°C to reduce the moisture content. It was stirred and separated using a magnetic separator to obtain magnetic sand. Subsequently, it was extracted by using 10 g of a sample and calcined at 800°C for 5 hours. The calcination process was conducted to reduce of Fe content by oxidation effect. These results were cooled at ambient temperature and separated using a magnetic separator to separate Fe$_2$O$_3$ as magnetic material and FeO.TiO$_2$ as non-magnetic material. The iron sand and FeO.TiO$_2$ were characterized by using XRD and XRF (PanAnalytical).

2.2. Pretreatment process using NaOH solution
The 500 g OPEFB powder was inserted into an explosion bench scale reactor containing a 10% NaOH (Sigma-Aldrich) solution of 2.5 L and heated at 150°C which the pressure of 4.0 bar for 30 min. The solution was inserted into the filter bag and pressed using a hydraulic press, filtered to produce a residue, and a black liquid containing black liquor of lignin.

2.3. Photodegradation of lignin using FeO.TiO$_2$ as catalyst
The photodegradation lignin was carried out using 20 mL lignin with the concentration of 300 ppm by the FeO.TiO$_2$ mass variation of 1.0; 2.0; and 3.0 g. Each of variation tested into reactor photocatalyst which containing the ultraviolet lamp (Black Light Blue (blb/uv-a)) and stirred with a hot plate magnetic stirrer (Cimarec Thermo Fisher Scientific). These treatments were conducted with degradation variation time for 0, 20, 30, 40, 50 and 60 min. The system filtered using filter paper and determined absorbance by UV-Vis spectrophotometer (JASCO V-730) at 280 nm. The same treatment was performed on 500 ppm and 700 ppm lignin solution.

2.4. Antibacterial activity test
The antibacterial activity test was performed by a well-diffusion method using 2 layers of solid Nutrient Borth (NB) medium and semi-solid NB. The solid NB was poured and solidified into a petri
dish as a base layer. The semi-solid NB was a liquid phase, added bacteria test as 1.0 mL then homogenous by Vortex-assisted at temperature ± 45-50°C. Subsequently, semi-solid NB was poured on the base layer and left to solidify, then molding to make wells. Lignin degradation solutions were added into wells as samples, negative control 10% NaOH, and positive control as amoxicillin. The media was inserted in the incubator for 24 hours at 37°C. After the incubation period, an inhibitory zone measurement was established to determine the results of an antibacterial inhibitory test.

2.5. Liquid Chromatography-Mass Spectrometry (LC-MS) characterization
The LC-MS was determined based on the antibacterial activity test results that high inhibitory bacterial i.e. *E. coli, S. aureus, S. typhi*, and *X. oryzae*. These results refer to concentration and time degradation variations due to the high inhibit the bacterial that assumed the phenolic group was performed in photodegradation lignin solutions.

3. Results and Discussion

3.1. Characterization of FeO.TiO$_2$

3.1.1. X-Ray Fluorescence (XRF). FeO.TiO$_2$ pre-oxidation was characterized by using XRF in order to obtain the components and levels after pre-oxidation. Figure 1 showed the composition ratio of iron sands (blue) and FeO.TiO$_2$ catalyst (red) after pre-oxidation.

![Figure 1. XRF data comparison between iron sands (blue) and FeO.TiO$_2$ catalyst (orange) after pre-oxidation](image)

Figure 1 shows the decrease of Fe content inversely proportional to the TiO$_2$ caused the calcination effect on FeO.TiO$_2$ mineral to influence of pre-oxidation process by using combustion reaction [27]. After the pre-oxidation process, the Fe content was decreased by 18.07%, while the total TiO$_2$ was increased to 5.13%. The pre-oxidation process has been obtained the 2.47 g FeO.TiO$_2$ as paramagnetic properties based on 10 g sample used.

3.1.2. X-Ray Diffraction (XRD). The XRD pattern exhibits the lattice dimension (d=crystal lattice) in FeO.TiO$_2$ structure. This method to obtain the high density and specific crystal. Figure 2 exhibits the comparison of XRD pattern from ilmenite and hematite. The FeO.TiO$_2$ pattern after pre-oxidation and compared by standard data on TiO$_2$ anatase, Fe$_2$O$_3$ (hematite), and FeO.TiO$_2$ that the iron sand after pre-oxidation samples contained Fe$_2$O$_3$ and FeO.TiO$_2$ minerals with rhombohedral crystal [28]. These
results were confirmed by comparison of Fe$_2$O$_3$ (JCPDS No. 33-0664) and FeO.TiO$_2$ (JCPDS No. 29-0733) databases in the crystal field 012 at 2 theta 24°.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{xrd_diagram.png}
\caption{Compatibility of XRD diffratogram (a) FeO.TiO$_2$ after pre-oxidation (b) TiO$_2$ anatase (JCPDS Card No. 21-1272) (c) hematite (JCPDS Card No. 33-0664) (d) ilmenite (JCPDS Card No. 29-0733)}
\end{figure}

According to Nurdin et al. and Morin et al. that the increasing of temperature showed the crystal change become of FeO.TiO$_2$ structure [21,28]. High stability crystal at 800°C formed rutile structure that occured the substituted material distortion of Fe atom presented at 2 theta 43°. This condition indicated the dominant component and affects the sample structure in the TiO$_2$ crystal [29,30]. The XRD pattern (Figure 2) showed that the sample was rutile structure formed because it does not show the anatase crystalline at 101 in 2 theta 25.1°. According to Xu et al. the photooxidation process using rutile crystal can provide good activity in the degradation process [31]. Rutile crystal provides a good degradation effect because it does not reduce the material properties just the difference in contact surface area.

3.2. Photooxidation of lignin using FeO.TiO$_2$ catalyst

This study, the FeO.TiO$_2$ material used to degrade at 300 ppm lignin by UV light irradiation. It was done to convert lignin to obtain the derivate monomer structure. The black liquor was containing a lignin compound will be reduced by FeO.TiO$_2$ photooxidation. We study the variation of time degradation and FeO.TiO$_2$ mass to obtain the optimal percent degradation. These results can be seen in table 1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Time (minute)} & \textbf{Catalyst (gram)} & \textbf{Initial concentration (ppm)} & \textbf{Final concentration (ppm)} & \textbf{\% Degradation} \\
\hline
10 & 0.1 & 228 & 189.33 & 16.959 \\
 & 0.2 & 228 & 182.000 & 20.175 \\
 & 0.3 & 228 & 158.667 & 30.409 \\
20 & 0.1 & 228 & 188.667 & 17.251 \\
 & 0.2 & 228 & 180.667 & 20.760 \\
 & 0.3 & 228 & 158.667 & 30.409 \\
\hline
\end{tabular}
\caption{Time and mass variations on lignin degradation in black liquor in 300 ppm black liquor}
\end{table}
Table 1 and Figure 3 show that the 300 ppm concentration lignin is a significant effect on the total of catalyst used in the photodegradation process. The 0.1 g FeO.TiO₂ has obtained the percent degradation of 16.959%, while the 0.3 g FeO.TiO₂ has increased the percent degradation of 30.409% for 10 minutes. In addition, at 20 minutes using 0.3 g catalyst showed an increase in the degradation rate with the degradation result of 30.409%. Figure 3 shows that optimization of lignin degradation occurs at 10 and 20 minutes at lignin concentration of 300 ppm. The smaller lignin concentrations indicates better catalyst performance in degradation process due to surface contact and the mass of catalyst which influences of percent degradation.

**Figure 3.** The percent degradation of lignin (a) the mass FeO.TiO₂ vs percent degradation, (b) time degradation vs percent degradation (0.1 g ; 0.2 g ; 0.3 g of FeO.TiO₂)

### 3.3. Antibacterial activity test

This study, we have the antimicrobial test by using 4 bacterial such as *E. coli*, *S. aureus*, *S. typhi*, and *X. oryzae*. The *E. coli* was tested using the degradation lignin solution at 300 ppm with 0.3 g FeO.TiO₂ mass showed that it has a strong inhibiting zone with a diameter of 20.67 mm. The data has been obtained as follows table 2.

| Sample code (FeO.TiO₂ mass – degradation time) | % degradation | Diameter zone (mm) | Information |
|-----------------------------------------------|---------------|-------------------|-------------|
| 0.3-10                                        | 30.409        | 15.67             | Weak        |
| 0.3-20                                        | 30.409        | 17.67             | Medium      |
| 0.3-30                                        | 31.579        | 20.67             | Strong      |
| 0.3-40                                        | 26.316        | 17.67             | Medium      |
| 0.3-50                                        | 26.316        | 19.33             | Medium      |
| 0.3-60                                        | 26.316        | 16.33             | Medium      |

Based on Table 2 can be seen that the 30 min degradation was generated 31.579% degradation lignin. It is explained that the effectively for lignin degradation process using FeO.TiO₂ as catalyst and active to inhibit *E.coli* bacterial. This causes the photodegradation process has exhibited the –OH radical as an antibacterial agent [32-34].

Meanwhile, the *S. aureus* bacterial test (Table 3) was conducted using 300 ppm lignin concentration. The same preparation which has been done on *E.coli* that the 0.3 g FeO.TiO₂ can inhibit *S. aureus* with diameter zone of 20.33 mm and 20.67 mm. The variation time degradation in 50 and 60 minutes assumed that the degradation still longtime needed to degrade and in order to obtain the derivate lignin. This phenomenon was caused by *S. aureus* which resistant stability on membrane cel.
It can be seen that percent degradation did not as a standard for high inhibitory bacteria. The decrease of percent degradation because of there is saturation on the catalyst surface.

**Table 3. Antibacterial activity result of *S. aureus* on 300 ppm lignin degradation**

| Sample code (FeO.TiO₂ mass – degradation time) | % degradation | Diameter zone (mm) | Information |
|-----------------------------------------------|---------------|--------------------|-------------|
| 0.3-10                                        | 30.409        | 16.67              | Medium      |
| 0.3-20                                        | 30.409        | 16.33              | Medium      |
| 0.3-30                                        | 31.579        | 17.67              | Medium      |
| 0.3-40                                        | 26.316        | 17.67              | Medium      |
| 0.3-50                                        | 26.316        | 20.33              | Strong      |
| 0.3-60                                        | 26.316        | 20.67              | Strong      |

**Table 4. Antibacterial activity result of *S. thypi* on 300 ppm lignin degradation**

| Sample code (FeO.TiO₂ mass – degradation time) | % degradation | Diameter zone (mm) | Information |
|-----------------------------------------------|---------------|--------------------|-------------|
| 0.3-10                                        | 30.409        | 14.67              | Weak        |
| 0.3-20                                        | 30.409        | 20.33              | Strong      |
| 0.3-30                                        | 31.579        | 16.67              | Medium      |
| 0.3-40                                        | 26.316        | 15.00              | Weak        |
| 0.3-50                                        | 26.316        | 11.67              | Weak        |
| 0.3-60                                        | 26.316        | 14.67              | Weak        |

Table 4 is *S. typhi* tested by a well-diffusion method showed that the 300 ppm concentration lignin by using 0.3 g FeO.TiO₂ also gives high inhibitory effect with diameter zone of 20.33 mm. The 20 min time degradation can be degraded 30.409% lignin which indicates that the high activity of lignin degradation compound compared with *S. aureus* activities. The *S. thypi* and *E. coli* have categorized as gram-negative that the membrane cell was consists of peptidoglycans (2-7 nm) between inner and outer films so it is categorized as a thin film layer membrane compared with gram-positive.

**Table 5. Antibacterial activity result of *X. oryzae* on 300 ppm lignin degradation**

| Sample code (FeO.TiO₂ mass – degradation time) | % degradation | Diameter zone (mm) | Information |
|-----------------------------------------------|---------------|--------------------|-------------|
| 0.3-10                                        | 30.409        | 17.67              | Medium      |
| 0.3-20                                        | 30.409        | 15.33              | Weak        |
| 0.3-30                                        | 31.579        | 19.67              | Medium      |
| 0.3-40                                        | 26.316        | 16.67              | Medium      |
| 0.3-50                                        | 26.316        | 11.67              | Weak        |
| 0.3-60                                        | 26.316        | 13.33              | Weak        |

The last tested by using *X. oryzae* bacteria showed that the 300 ppm concentration lignin was active with diameter zone is 17.67 mm. These results prove that the degradation able optimization process to form simple phenolic compounds of lignin. The 0.3 g FeO.TiO₂ be able to degrade lignin is 30.409% lignin with a short time at 10 min. Based on the antibacterial tests show the 300 ppm concentration lignin can be able to degrade with percent degradation of ±30% using 0.3 g FeO.TiO₂. These results were characterized by LC-MS to prove the lignin derivated containing a phenolic group. According to Nurdin et al. that the inhibitory zone formed by lignin degradation contains phenolic derivatives which can decompose cell membranes from bacteria [26].

3.4. **Determination of lignin derivate by using LC-MS**

The 300 ppm lignin concentration was analyzed using LC-MS which active against bacterial tests. It can be seen (Figure 4a) that the LC spectrum has exhibited 4 dominant spectrum peaks with a

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*Note: The table and text content is extracts from a research paper and has been formatted for readability.*
retention time (Rt) of 0.78; 5.71; 22.10, and 22.46. The LC spectrum shows the samples preparation were good separate and no various impurity spectra. This situation occurs due to a high extraction method using 10% NaOH for lignin solubilizing. Based on LC spectrum no any impurity peaks both solvent or inorganic material detected.

Figure 4. The LC-MS spectrum of 300 ppm concentration lignin, a) Liquid Chromatography, and b) Mass Spectroscopy

This proves the chemical compound has been isolated as good purity from other impurities. Based on each result of the retention time and the LC peaks then analyzed by using MS showed that this compound has good separated and stability fragmentation molecule. Figure 4b shown in MS spectrum that the form matching to the reference at a concentration of 300 ppm with retention time of 5.71. This indicates the presence of C_{10}H_{12}O_{3} as Coniferyl Alcohol compound with m/z value of 179.081. According to Morin et al. that the retention time of 5.73 with a molecular weight of 179.090 is the Coniferyl Alcohol compound formed [28].

4. Conclusions

We successfully applied the degradation of lignin using FeO.TiO_{2} catalyst as extracted from iron sand from Southeast Sulawesi, Indonesia to obtain derivate of lignin compound. Based on the XRF and XRD data indicated that the iron sand contained Fe_{2}O_{3} (hematite) and FeO.TiO_{2} minerals with the highest dominant of Fe content in the sample. The lignin photodegradation showed that the lignin-derived compound has been obtained as Coniferyl Alcohol (C_{10}H_{12}O_{3}). Antibacterial activity test against 4 bacterial samples i.e. *E. coli*, *S. aureus*, *S. typhi*, and *X. oryzae* shows that each test has good activity in inhibiting bacteria with a range diameter zone of ±20 nm. This study provides that lignin-derived compounds from OPEFB can be used as a natural antibacterial.

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