Short Paper

Optimization of Particle Size, Moisture Content and Reaction Time of Oil Palm Empty Fruit Bunch Through Ozonolysis Pretreatment

Efri MARDAWATI, Herlin HERLIANSAH †, Edy SURYADI, In In HANIDAH, Imas SITI SETIASIH, Robi ANDOYO, Een SUKARMINAH, Mohammad DJALI, Tita RIALITA, and Yana CAHYANA

(Received September 19, 2018)

Ozone is a powerful oxidant and is reactive toward lignin. Ozone can be used as an oxidant in pre-treatment process of lignocellulose material without producing any toxic residues nor making structural changes in cellulose and hemicellulose during ozonolysis process. This study aims to evaluate the effectiveness of ozone for the delignification of oil palm empty fruit bunch (OPEFB). The effect of 20, 40 and 60 mesh particle size, 30, 40 and 50% moisture content, and 30, 45 and 60 minute reaction time as well as their interactions, on lignin degradation, holocellulose content and reducing sugar concentration is investigated using response surface methodologies (RSM) with Design Expert 10. The total number of pre-treatment variations is determined by Box Behnken. The results show that ozonolysis is an effective method for delignifying lignin up to 63.86% and for increasing cellulose up to 40.95%. The ozonolysis process is able to degrade lignin and hemicellulose without decreasing the cellulose. The optimum condition of lignin degradation after the ozonolysis pre-treatment occurs at the condition with 40 mesh particle size, 50% moisture content and 30 minute reaction time. Enzymatic hydrolysis of OPEFB which has underwent ozonolysis pre-treatment can increase the concentration of reducing sugar. The optimum condition for lignin degradation is 42.42% and the reducing sugar concentration of 0.40 g / L is determined by the condition with 40 mesh particle size, 30% moisture content and 39.6 minute reaction time.

Key Words
Biomass pretreatment, Lignin degradation, Oil Palm Empty Fruit Bunch, Ozonolysis, Reducing Sugar Concentration

1. Introduction

Oil Palm Empty Fruit Bunch (OPEFB) is one of the most abundant agricultural products available in Southeast Asia, especially in Indonesia and Malaysia 1). About 20% of the weight of oil palm fresh fruit is OPEFB 2). OPEFB itself contains Lignocellulosic components including 43-43.47% cellulose, 22.93-23.67% hemicellulose and 21.28-22.10% lignin. The high amount of cellulose contained makes it possible to utilize OPEFB to be hydrolyzed into glucose. Cellulose will be hard to hydrolyze into simple sugars if there is no pretreatment process done on the biomass. The pretreatment process itself is a lignin delignification process of cellulose and hemicellulose, thus, it will make the scarification process easier to perform 3). The purpose of this pretreatment process is to reduce cellulose crystallinity properties, to increase substrate surface area and porosity, and to destroy cellulose heterogeneous structures 4).

The pretreatment process of OPEFB can be done in three methods, namely physical, chemical and biological methods 6). According to Sun, Y. et al. 7), pretreatment process does not necessarily require high costs, does not affect carbohydrate degradation, does not produce by-products that can inhibit hydrolysis and fermentation processes, and can produce sugar at low enzyme consumption. In addition, it can be done at low concentrations, low temperature, low pressure, and fast reaction.

As for the ozonolysis process, it is one of the pretreatment processes that can be used for delignification. According to Mardawati, E. et al. 8), ozone can be used effectively to delignify without producing toxic residues nor making structural changes in cellulose and hemicellulose.
during the ozonolysis process. Ozone is a powerful oxidant and highly reactive toward lignin due to its conjugated double-bonds and functional groups with high electron densities. Ozone can decompose into oxygen, therefore, it is very safe to throw it into the environment, and its filtrate from the washing stage can be reused for lignin recovery. Inhibitor products resulted from ozonolysis process are different from each other, depending on the reactivity of the lignin functional group. The ozonolysis process of bagasse carried out by Travaini, R. et al. finds inhibitors resulted including oxalic acid, formic acid, and levulinic acid, yet finds no furfural and HMF compounds.

The ozonolysis pretreatment is influenced by several factors, including particle size, water content and reaction time. The particle size corresponds to the surface area available during the reaction. Meanwhile, the moisture content of the raw material assists in a three-step reaction, namely the transition from ozone as a gas phase to a mobile water phase to react with biomass. When the ozonolysis pretreatment, ozone affecting wet biomass dissolved by the water contained in the biomass thus releasing oxidation products and enabling minor lignin chain termination reactions. As for the reaction time, it is closely related to ozone concentration and flow rate during the reaction. The duration of ozonolysis reaction time has an influence on reducing sugar concentration.

This paper presents the study of optimization of particle size, moisture content and reaction time of ozonolysis pretreatment for lignin delignification, holocellulose content, and reducing sugar concentrations from ozone-treated OPEFB.

2. Experiment

The material used in this study is OPEFB with mesh size 20, 40 and 60, obtained from oil palm plantation of PT. Perkebunan Nusantara VIII Kebun Cikasungka in Cigudeg, Bogor, West Java. Chesson method was used for analyzing the composition of lignocellulose. The composition of OPEFB can be seen on Table 1.

The experiment of ozonolysis pretreatment is designed by a PC software (Design Expert version 10). A three-level factorial with Box-Behnken design (BBD) is used for investigating the effects of process variables to obtain optimal values. Three independent process variables, namely particle size ($X_1$), moisture content ($X_2$), and reaction time ($X_3$) are selected. Meanwhile, the main response involves the condition of holocellulose, lignin degradation and reducing sugar concentration. The level and range of process variables are summarized in Table 2.

This experiment of ozonolysis pretreatment refers to the study by Wan Omar, W. N. N. et al., in which 10 g of OPEFB whose particle size and water content have been conditioned in accordance to the design of experiment is included in 1 L Erlenmeyer Flasks, the outlet hose of the ozonizer was inserted, and the flasks are tightly closed. This process was carried out for 30, 40 and 60 minute according to the independent variables in Table 2. After the ozonolysis process was completed, the sample is then immersed with 100 mL of 5% NaOH at room temperature for 60 minute. Furthermore, the sample is filtered and rinsed using hot aquades to be neutral. The neutral sample is dried by the oven at 105°C for 50 minute. In this study, the oxygen flow rate is 10 L/minute and the ozone concentration is 16 ppm from iodometric method. Then, a testing is performed on the oven-dried OPEFB lignocellulose using Chesson method. Fig. 1 shows a schematic diagram of the OPEFB ozonolysis reactor set up for this study, which consists of ozone generator (Model OZ-10G, Luso Ozonizer, China), oxygen tank, oxygen flow rate, ozone pipe and reaction vessel. The oxygen set by the flow rate of 10 L/minute is flowed to the ozone generator which converts oxygen to ozone. The ozone that comes out of the ozone generator is flowed towards flasks which contains biomass.

![Fig. 1 Schematic illustration of the ozone generation and reactor system used in this study. Operating conditions: reactor volume = 1 L, oxygen flow rate = 10 L/minute and ozone concentration = 16 ppm](image)
ozone concentration was previously tested using iodometric method. Flasks was closed tightly using a stopper.

Hydrolysis enzymatic was performed on ozone-treated OPEFB. Two grams of oven-dried OPEFB is suspended in 250 mL Erlenmeyer flasks in 50 mL citrate buffer pH of 4.8, and 5 mL cellulase from Institut Teknologi Bandung (1947,368 FPU/mL). These flasks are placed in an incubator shaker at 50°C and 150 rpm for 48 hours. Furthermore, reducing sugar is then tested using DNS method.

3. Results and Discussion

3.1 Effect of Pretreatment Ozonolysis On Lignin Degradation and Holocellulose Content

Based on Table 3, the effect of ozonolysis pretreatment on OPEFB causes a decrease in lignin and hemicellulose content, as well as an increase in cellulose content. Both the decrease in lignin and the increase in cellulose content has been explained by Lee, Y. et al. and Schultz-Jensen, N. et al. The optimum lignin delignification occurs on 40 mesh particle size, 50% moisture content and 30 minute reaction time with lignin delignification rate of 63.86%. The following scheme presents the optimization of lignocellulose content model:

\[
Y_1 = 41.43 - 0.90 A - 0.08 B - 0.42 C - 5.27 \times 10^{-4} AB - 3.99 \times 10^{-3} AC + 7.38 \times 10^{-3} BC + 0.01 A^2 - 4.61 \times 10^{-3} B^2 + 3.55 \times 10^{-3} C^2 \quad (1)
\]

\[
Y_2 = -15.33 + 1.31 A + 0.58 C - 1.86 \times 10^{-3} AB + 5.28 \times 10^{-5} AC - 1.59 \times 10^{-3} BC + 0.01 A^2 + 4.59 \times 10^{-3} B^2 - 3.55 \times 10^{-3} C^2 \quad (2)
\]

\[
Y_3 = 20.35 + 1.075 A + 0.67 B + 0.57 C + 0.017 AB - 5.37 \times 10^{-3} AC + 1.59 \times 10^{-3} BC - 0.02 A^2 - 0.019 B^2 + 3.35 \times 10^{-3} C^2 \quad (3)
\]

Description:
- \( Y_1 \) = lignin content
- \( Y_2 \) = hemicellulose content
- \( Y_3 \) = cellulose content
- \( A \) = particle size (mesh)/ (X₁)
- \( B \) = water content (%)/ (X₂)
- \( C \) = reaction time (minute)/ (X₃)

Based on the analysis of variance (ANOVA) of the three variables, the particle size is the most influential variable in relation to lignocellulose content, with the p-value probability > F less than 0.05. The determination coefficient (R²) of each response variable is 84% for lignin content with pure error 4.54 and 97% for hemicellulose and cellulose content with pure error 0.55. These values mean that in relation to the decrease in lignin content, particle size has an effect of 84% while the rest is influenced by other factors, and hemicellulose and cellulose levels do likewise.

Fig. 2 shows the effect of particle size, moisture content and reaction time on ozonolysis pretreatment. According to Fig. 2, the lignin content will decrease optimally in the mesh with particles size of 40 and non-optimally in the mesh with particles size of 60. This condition is similar to Bule, M. V. et al. Whereas in 60 mesh particle size, the lignin levels in fiber range from

| Design of experiment | Particle size (mesh) (X₁) | Water Content (%) (X₂) | Reaction Time (minute) (X₃) | Hemicellulose content (%) | Cellulose content (%) | Lignin content (%) | Lignin degradation (%) | Reducing Sugar Concentration (g/L) |
|----------------------|---------------------------|------------------------|-----------------------------|--------------------------|----------------------|---------------------|------------------------|----------------------------------|
| 1                    | 40                        | 40                     | 45                          | 16.07                    | 59.24                | 12.32               | 45.93                  | 0.39                             |
| 2                    | 40                        | 40                     | 45                          | 17.35                    | 54.78                | 8.313               | 63.52                  | 0.46                             |
| 3                    | 20                        | 40                     | 30                          | 10.02                    | 55.79                | 16.64               | 26.97                  | 0.16                             |
| 4                    | 20                        | 30                     | 45                          | 12.50                    | 59.33                | 17.52               | 23.09                  | 0.20                             |
| 5                    | 40                        | 50                     | 60                          | 14.58                    | 52.23                | 14.23               | 37.56                  | 0.59                             |
| 6                    | 20                        | 40                     | 60                          | 12.40                    | 58.49                | 14.75               | 23.41                  | 0.36                             |
| 7                    | 40                        | 40                     | 45                          | 16.40                    | 51.01                | 13.77               | 39.55                  | 0.36                             |
| 8                    | 40                        | 50                     | 30                          | 15.06                    | 52.80                | 8.23                | 63.86                  | 0.46                             |
| 9                    | 40                        | 40                     | 45                          | 15.72                    | 51.52                | 12.77               | 43.97                  | 0.42                             |
| 10                   | 40                        | 40                     | 45                          | 13.43                    | 58.29                | 10.75               | 52.82                  | 0.40                             |
| 11                   | 40                        | 30                     | 30                          | 16.64                    | 53.24                | 11.83               | 48.07                  | 0.33                             |
| 12                   | 20                        | 50                     | 45                          | 13.70                    | 56.87                | 13.40               | 41.17                  | 0.36                             |
| 13                   | 60                        | 30                     | 45                          | 9.58                     | 27.36                | 20.76               | 8.91                   | 0.20                             |
| 14                   | 60                        | 40                     | 60                          | 6.82                     | 30.35                | 17.44               | 23.47                  | 0.16                             |
| 15                   | 40                        | 30                     | 60                          | 17.11                    | 53.62                | 13.40               | 41.20                  | 0.51                             |
| 16                   | 60                        | 50                     | 45                          | 9.29                     | 33.21                | 16.21               | 28.84                  | 0.22                             |
| 17                   | 60                        | 40                     | 30                          | 10.78                    | 34.09                | 21.41               | 6.04                   | 0.15                             |
Fig. 2 3D surface of desirability function on lignocellulose content
The hemicellulose content will decrease drastically in 60 mesh particle size. Hemicellulose itself is a water-soluble or alkali-soluble lignocellulose component and is susceptible to degradation at high temperatures. In this study, the degradation of hemicellulose is considered to occur due to several causes. First, hemicellulose is reduced by ozone after the redox process in lignin occurs. In ozonolysis pretreatment of aspen powder, hemicellulose is the second component undergoing oxidation after lignin. Second, after the process of gazing, the sample is immersed in 5% NaOH at room temperature for 60 minute, then neutralized by washing it using hot water. It is presumed that in this process, hemicellulose participates and dissolves in NaOH and then wasted when washed using hot water. Similarly, in the ozonolysis pretreatment of wheat straw and rye, the immersion of NaOH can degrade hemicellulose up to 50%. Whereas in terms of cellulose content, in 20-40 mesh particle size, cellulose experiences a high increase. However, for these three components, neither the moisture content nor the reaction time has a significant effect.

3.2 Effect of Ozonolysis Pretreatment on Enzymatic Hydrolysis

Without ozonolysis pretreatment, OPEFB hydrolyzate produces reducing sugars of 0.08 g/L, while with it, the reducing sugars resulted increases. Based on Table 3, under the condition of 40 mesh particle size, moisture content 50% and reaction time 60 minute, the reducing sugar concentration produced is 0.59 g/L. This condition is considered the optimum condition for producing reducing sugars since the result is the highest of all. As for the optimization of reducing sugar concentration model, it is presented by the following scheme:

\[
Y_4 = -0.52 + 0.05 A - 0.03 B + 8.81 \times 10^{-3} C - 1.70 \times 10^{-4} AB - 1.66 \times 10^{-4} AC - 8.65 \times 10^{-4} BC - 5.36 \times 10^{-4} A^2 + 5.23 \times 10^{-4} B^2 - 6.30 \times 10^{-4} C^2
\]  

(4)

Description:

- \( Y_4 \): Reducing sugar concentration (g/L)

The results of the analysis of variance (ANOVA) indicate that the most influential variables on the reducing sugar concentration are A (particle size), B (moisture content), C (reaction time), A^2 and B^2. The coefficient of determination (R^2) is 97% meaning that the influence of the independent variables on the dependent variable changes 97% of the concentration of reducing the sugar, while the remaining 3% is influenced by other factors outside the independent variables of this study. Pure error for the reducing sugar concentration is 1.38 \times 10^{-3}.

According to Fig. 3, the optimum condition of reducing sugars is when the particle size is 40 mesh. Moreover, the ozonolysis pretreatment condition that produces the optimum reducing sugars is the one with 40 mesh particle size. The increase of reducing sugars is influenced by the increase of moisture content and reaction time. In the study by Wan Omar, W. N. N. et al., the more the reaction of ozonolysis occurred, the lesser the reducing sugars produced. It is likely to be caused by cellulose content degraded during the pretreatment process. In this study, the ozone concentrations used are lower so that the effect resulted on cellulose degradation is very small. The enzymatic hydrolysis process will be hard to perform if components blocking the penetration of cellulose enzymes such as lignin are not removed. After the ozonolysis pretreatment, the hydrolyzed OPEFB substrate is capable of producing higher glucose than OPEFB which is not given preliminary treatment. It means that the ozonolysis pretreatment is able to degrade lignin, therefore, hydrolysis processes can occur.

Based on Table 2, in this study, the ozonolysis pretreatment degrades the lignin and increases the reducing
sugar concentration. The experiment number 8 (40 mesh particle size, 50% moisture content, and 30 minute reaction time) is the experiment with the highest level of lignin degradation. The lignin degradation achieved is 63.86%, and the reducing sugar concentration resulted after hydrolysis is 0.46 g/L.

3.3 Optimization of Ozonolysis Pretreatment

The optimum values of the processing results will be shown through the desirability function. In terms of solution taken, the solution with the desirability value nearest 100% is expected to be the best solution. The selection of these solutions is based on feasibility study. The best optimum point has a desirability value of 96.10%. Prediction values are presented in Table 4.

4. Conclusion

The effect of ozonolysis pretreatment on OPEFB has been investigated and optimized. The ozonolysis pretreatment degrades the lignin and increases the reducing sugar concentration. The response surface methodology (RSM), based on Box Behnken, indicates that the particle size is important for the degradation of lignin and for the increase of reducing sugar. Both lignin degradation and reducing sugar concentration are optimized simultaneously by the desirability function. As a result, the optimum condition for lignin degradation is 42.42% and the reducing sugar concentration of 0.40 g/L is determined by the condition of 40 mesh particle size, 50% moisture content and 30 minute reaction time.

Acknowledgment

Authors are grateful to Universitas Padjadjaran for funding this study through Academic Leadership Grant Program (1-I-6 ALG Program).

Table 4  Validation of the predicted model at different condition

| Particle size  | Water content (%)(X1) | Reaction time (minute)(X2) | Lignin content (%) (X3) | Hemicellulose content (%) | Cellulose content (%) | Lignin degradation (%) | Reducing sugar (g/L) |
|---------------|------------------------|-----------------------------|--------------------------|---------------------------|----------------------|-----------------------|----------------------|
| 39.79(40)     | 30                     | 39.6                        | 13.12                   | 12.05                     | 51.34                | 42.42                 | 0.40                 |
|               |                        |                             | 12.80                   |                           |                      |                       |                     |

a = observed; b = predicted model from Design Expert 10

References

1) Yano, S.; Murakami, K.; Sawayaama, S.; Imou, K.; Yokoyama, S., J. Jpn. Inst. Energy, 88, 923-926 (2009)
2) Mohammad, N.; Alam, M. Z.; Kabbashi, N. A.; Ahsan, A., Resour., Conserv. Recycle, 58, 69-78 (2012)
3) Mardawati, E.; Werner, A.; Bley, T.; Kresnowati, M.; Setiadi, T, J. Jpn. Inst. Energy, 93, 973-978 (2014)
4) Tan, L.; Yu, Y.; Li, X.; Zhao, J.; Qu, Y.; Choo, Y-M.; Loh, S-K., Bioresour. Technol., 135, 275-282 (2013)
5) Talebnia, F.; Karakashev, D.; Angelidaki, I., Bioresour. Technol., 101, 4744-4753 (2010)
6) Chen, H.; Liu, J.; Chang, X.; Chen, D.; Xue, Y.; Liu, P.; Lin, H.; Han, S., Fuel Process. Technol., 160, 196-206 (2017)
7) Sun, Y.; Cheng, J., Bioresour. Technol., 83, 1-11 (2002)
8) Mardawati, E.; Windarningsih, F.; Andoyo, R.; Rialita, T.; Hanidah, I.; Sukarminah, E.; Djalil, M.; Cahyana, Y.; Nur, M.; Setiasih, I. S., The Effect of Ozonolysis Pretreatment of Xylose Hydrolysis Yield of Oil Palm Leaves and Petioles., Prep. 3rd Asian Conf. Biomass Sci., OC2, Jan, 49-55, 2016, Niigata, Japan
9) Al Jibouri, A. K. H.; Turcotte, G.; Wu, J.; Cheng, C., Energy Sci. Eng., 3, 541-548 (2015)
10) Wan Omar, W. N. N.; Amin, N. A. S., Ind. Crop. Prod., 85, 389-402 (2016)
11) Travaini, R.; Martin-Juárez, J.; Lorenzo-Hernando, A.; Bolado-Rodríguez, S., Bioresour. Technol., 199, 2-16 (2016)
12) Travaini, R.; Otero, M. D. M.; Coca, M.; Da-Silva, R.; Bolado, S., Bioresour. Technol., 133, 332-339 (2013)
13) Travaini, R.; Marangon-Jardim, C.; Colodette, J. L.; Morales-Otero, M.; Bolado-Rodriguez, S., 7 Ozonolysis in Pretreatment of Biomass Processes and Technologies, Eds. Pandey, A.; Negi, S.; Binod, P.; Larroche, C., Elsevier, 2014
14) Neely, W. C., Biotechnol. Bioeng., 26, 59-65 (1984)
15) Cogo, E.; Albet, J.; Malmary, G.; Coste, C.; Molinier, J., Chem. Eng. J., 73, 23-28 (1999)
16) Barros, R. da R. O. de; Paredes, R. de S.; Endo, T.; Da Silva Bon, E. P.; Lee, S. H., Bioresour. Technol., 136, 288-294 (2013)
17) García-Cubero, T.; González-Beníto, G.; Indacochea, I.; Coca, M.; Bolado, S., CIGR J., 16, 151-156 (2014)
18) Datta, R., Biotechnol. Bioeng., 23, 2167-2170 (1981)
19) Wan Omar, W. N. N.; Amin, N. A. S., Ind. Crops Prod., 85, 389-402 (2016)
20) Masschelein, W. J., J. Intl. Ozone Association, 20, 191-203 (1998)
21) Adney, B.; Baker, J., Measurement of Cellulase Activities Laboratory Analytical Procedure (LAP)
22) Lee, Y.; Chung, C.; Day, D. F., *Bioresour. Technol.*, **100**, 935-941 (2009)
23) Schultz-Jensen, N.; Kádár, Z.; Thomsen, A. B.; Bindslev, H.; Leipold, F., *Appl. Biochem. Biotechnol.*, **165**, 1010-1023 (2011)
24) Bule, M. V.; Gao, A. H.; Hiscox, B.; Chen, S., *J. Agric. Food Chem.*, **61**, 3916-3925 (2013)
25) Mamleeva, N. A.; Autlov, S. A.; Bazarnova, N. G.; Lunin, V. V., *Pure Appl. Chem.*, **81**, 2081-2091 (2009)
26) Mardawati, E.; Kresnowati, M.; Purwadi, R.; Bindar, Y.; Setiadi, T., *International Journal on Advanced Science, Engineering and Information Technology*, **8**(6), 2539-2546 (2018)
27) Mardawati, E.; Parlan; Rialita, T.; Nurhadi, B., *IOP Conf. Ser.: Earth Enviro Sci.*, **141**(012018), 1-10 (2017)