Enzymatic hydrolysis of oil palm empty fruit bunch to produce reducing sugar and its kinetic

**Hidrolisis enzimatik tandan kosong kelapa sawit untuk menghasilkan gula pereduksi dan kinetikanya**

Vera BARLIANTI*, DelianaDAHNUM, MURYANTO, EkaTRIWAHYUNI, 
YosiARISTIAWAN&YanniSUDIYANI

Research Centre for Chemistry, Indonesian Institute of Sciences (LIPI) 
Kawasan Puspiptek Serpong, Tangerang Selatan, Indonesia

Diterima tanggal 19 Desember 2014/disetujui tanggal 5 Maret 2015

**Abstrak**

Sebagai salah satu Negara penghasil minyak kelapa sawit mentah (CPO), Indonesia juga menghasilkan tandan kosong kelapa sawit (TKKS) dalam jumlah besar. TKKS terdiri dari tiga komponen utama, yaitu selulosa, hemiselulosa, dan lignin. Pengolahan awal TKKS secara alkimindik iki dengan hidrolisis TKKS secara enzimatik menggunakan kombinasi enzim selulasa dan β-glukosidase akan menghasilkan gula-gula yang mudah difermentasi. Penelitian ini bertujuan untuk mempelajari pengaruh konsentrasi substrat, konsentrasi enzim, dan suhu selama proses hidrolisis berlangsung. Hasil yang diperoleh menunjukkan bahwa konsentrasi gula maksimum (194,78 g/L) dicapai pada konsentrasi TKKS 20% (b/v), konsentrasi campuran enzim yang terdiri dari selulasa dan β-1,4-glukosidase sebesar 3,85% (v/v), dan suhu 50°C. Perbandingan antara selulasa dan β-1,4-glukosidase adalah 5:1 dengan masing masing aktivitas enzim sebesar 144.5 FPU/mL dan 63 FPU/mL. Hasil penelitian juga menunjukkan bahwa model kinetika yang sesuai untuk proses hidrolisis TKKS secara enzimatik adalah model kinetika Shen dan Agblevor dengan reaksi side aktivasi enzim orde satu. Hasil ini mendukung studi kelayakan ekonomi dalam pemanfaatan TKKS untuk produksi bioetanol.

[Kata kunci:Tandan kosong kelapa sawit, hidrolisis enzimatik, kinetika, selulasa, gula pereduksi]

**Abstract**

As one of the crude palm oil producers, Indonesia also produces empty fruit bunches (EFB) in large quantities. The oil palm EFB consists of cellulose, hemicellulose and lignin. Alkaline pretreatment of EFB, followed by enzymatic hydrolysis of cellulose using combination of cellulase and β-glucosidase enzymes produce fermentable sugars. This paper reported the effects of substrate loading, enzyme concentration, and temperature of hydrolysis process on reducing sugar production. The maximum sugar concentration (194.78 g/L) was produced at 50°C using 20% (w/v) EFB and 3.85% (v/v) mixed enzymes of cellulase and β-1,4 glucosidase in volume ratio of 5:1 (v/v), with enzyme activity of 144.5 FPU/mL and 63 FPU/mL, respectively. The results also showed that the suitable kinetic model for enzymatic hydrolysis process of oil palm EFB follow Shen and Agblevor model with first order of enzyme deactivation. These results support the economic feasibility study in utilization of EFB of oil palm for bioethanol production.

[Key Words : Empty fruit bunch, enzymatic hydrolysis, kinetics, cellulase, reducing sugar]

**Introduction**

Indonesia is one of oil palm (Elaeis guineensis) producers in the world. In 2012 area of oil palm plantation reached 9.074.621 ha (Ditjenbun, 2014a) with CPO production as much as23,521.071 tons (Ditjenbun, 2014b). Along with increasing of CPO production, empty fruit bunches (EFB) as one of solid waste from palm oil mill will be available in large quantities. Treatment of one ton of fresh fruit bunchin palm oil industry will supply EFB as 0.23 ton (Yunus et al., 2010). The oil palm EFB consist of cellulose (37.62%), hemicellulose (14.62%), and lignin (31.68%). The rest are extractive materials and ash (Styarini et al., 2012). The common utilization of EFB was not maximum yet, in general EFB was used as soil mulching and co-composting with treated palm oil mill effluent (POME) in palm oil plantation (Baharuddin et al., 2009). One effort to give added value on EFB is converting cellulose (main component of EFB) to fermentable sugar that can be changed to other products easily, like bioethanol (Sun& Cheng, 2002).

Cellulose can be hydrolysed enzimately using cellulase enzyme, a complex enzyme that consists of exocellulase or exobiohydrolyase, endocellulase or endo-β-1,4-glucanase and β-1,4-glucosidase endocellulase. Enzymatic hydrolysis process still hampered by slow reaction rate that

*) Penulis korespondensi: vera.barlianti@gmail.com
caused by compact structure of lignocellulose. The compact structure prevent enzyme penetration to active site of cellulose (Banerjee et al., 2010), therefore it is necessary to do a pretreatment process to liberate cellulose from lignocelluloses matrix. Generally, pretreatment processes were classified into physical pretreatments, chemical pretreatments, and biological pretreatments. Different type of lignocellulosic materials require different type of pretreatment process according to their characteristics. The alkaline pretreatment was effective to remove lignin and acetyl group, but the process was relatively slow. This process was suitable for biomass from herbs or agricultural residue, but not for wood with high lignin content (Banerjee et al., 2010).

The pretreated lignocelluloses still have a complex structure and composition. Actually they require the different enzymes and an appropriate condition for complete hydrolysis. Therefore it was needed to simplify analysis of effective enzymatic hydrolysis on different operation condition, and studying the hydrolysis reaction kinetics was the answer to meet the needs.

Based on fundamental approach and methodology, kinetic models of lignocellulososes hydrolysis were classified to four classes namely empirical models, Michaelis-Menten based models, models accounting for adsorption, and models developed for soluble substrates (Bansal et al., 2009). Some research of this topics were hydrolysis kinetic of food waste based on adsorption mechanism (Kim et al., 2005), hydrolysis kinetic of lignocelluloses based on fractal kinetic analysis (Yao et al., 2011), and so on.

This paper reported the effects of substrate loading (percentage of oil palm EFB in a given reaction volume), reaction temperature, and enzyme concentration on hydrolysis process of EFB to produce reducing sugar and its kinetic. The EFB had been pretreated using 10% of sodium hydroxide solution previously.

**Materials and Methods**

**Raw Material**

The EFB were from Pandeglang, Indonesia. The EFB were sun-dried for several days, and chopped to form small pieces of EFB. Then the EFB were ground using grinder and sieved to get 3mm of EFB. The EFB's composition was determined by triplicates data using standard Biomass Analytical Procedures methods provided by National Renewable Energy Laboratory (NREL).

Enzymes used in hydrolysis of lignocelluloses were cellulase and β-1,4, glucosidase from Novozyme with enzyme ratio of 5 : 1. The activities of cellulase and β -1,4, glucosidase based on measurement were 144.5 FPU/mL, and 63 FPU/mL respectively. The chemicals used in this study had analytical grade.

**Pretreatment**

Pretreatment process was carried out by alkaline method using 10% of sodium hydroxide solution. The process was held at 150°C and 4-7 kg/cm² for 30 minutes. As much as 50 kg of EFB were fed into the reactor containing 250 L of sodium hydroxide solution. At the end of process, EFB were separated from liquid by belt press separation. The next step was washing of EFB by tap water in washing tank one and washing tank two repeatedly until it reached a neutral. Pretreated EFB were dried at 40°C for 24 hours. A part of pretreated EFB was used for analysis of the composition of EFB after pretreatment, and the others were ready for hydrolyzed and other research activities.

**Enzymatic Hydrolysis**

Enzymatic hydrolysis was carried out using the Erlenmeyer flasks of 500 mL with 200 mL of working volume at pH 4.8. The Erlenmeyer flasks containing of EFB substrate, enzymes, and citrate buffer was held in shaking incubator at 150 rpm. Three different concentrations of substrate (10%, 15%, and 20% (w/v)), and three different enzyme concentrations (2.574%, 3.852% and 5.148% (v/v)) were tested. The experiment was held at 32°C, 40°C, and 50°C. The sampling was done every 4 hours on first day, and every 24 hours on second and third days. Each sample was always put in boiling water for two minutes to deactivate the enzymes prior to reducing sugar concentration measurement.

**Determination of reducing sugar concentration**

Reducing sugar concentration during the process was determined by Somogyi-Nelson method. 1 mL of sample was placed into a reaction tube, and then 1 mL of Nelson reagent was added into the tube. The reaction tube was put in boiling water for 20 minutes. After the temperature of tube decreased to room temperature, 7 mL of aquadest and 1 mL of Arsenomolybdic reagent were added, and the tube was then shaken until all Cu₂O deposit was dissolved. The absorbance of the sample was analyzed by UV-Vis spectrophotometer at a wavelength of 520 nm. Meanwhile, glucose (one of reducing sugar’s components) concentration during the process was analyzed by high performance liquid chromatography (HPLC) method.

**Results and Discussion**

**Effect of substrate loading and reaction temperature**

The production of reducing sugar tends to increase in line with substrate loading from 10 to 20% (w/v) (Figure 1) in all reaction temperatures tested in this study. The highest sugar concentration (194.78 g/L) was achieved when 20% (w/v) EFB was applied. It illustrated that substrate loading was
an important factor in enzymatic hydrolysis of lignocellulose materials. The sugar yield showed the opposite tendency while the substrate loading increases (Figure 2). This tendency might be caused by increasing viscosity of the reaction mixture at higher substrate loading, therefore decreasing the effect of stirring and hamper the enzyme to reach the active site of cellulose. The influence of viscosity could also decrease the initial reaction rate (see Figure 1b and 1c). It was shown that at the temperature below 50°C, the initial reaction rate at 20% (w/v) of substrate loading was slower than others. Another cause was possibility of product inhibition by high sugar concentration to deactivate the enzyme activities (Musatto et al., 2008). The sugar yield determination was done with respect to the composition of pretreated EFB as seen in Table 1.

Figure 2. illustrated the positive correlation between sugar concentration and substrate loading and the negative correlation between sugar yield and substrate loading, and it was obtained an optimal substrate loading at 18% (w/v) of EFB. This substrate loading would give sugar concentration and sugar yield around 176.034 g/L and 1.160 g/g (cellulose + hemicellulose) respectively. The effects of substrate loading in enzyme hydrolysis of lignocelluloses materials were also performed on corncob (Chen et al., 2007), barley straw (Rosgaard, 2007), and more. Rosgaard (2007) studied the relationship between substrate loading and viscosity of the reaction. For optimizing the hydrolysis reaction of barley straw with respect to glucose concentration and glucose yield, an optimal substrate loading at the beginning of the reaction was 12.5% (w/w) DM of barley straw.

Figure 3. showed the increase of reducing sugar concentration along with increasing of hydrolysis temperature. The maximum sugar concentration was obtained at 50°C. This happened also on sugarcane bagasse hydrolysis (Mahamud & Gomes, 2012), or food waste hydrolysis (Kim et al., 2005). It showed that increasing temperature could accelerate the reaction rate by improving performance of the enzyme functions to produce reducing sugar. The best hydrolysis temperature for these enzymes was 50°C.

Table 1. The composition of pretreated EFB.

| Component     | Composition (%) |
|---------------|-----------------|
| Cellulose     | 77.5            |
| Hemicellulose | 6.83            |
| Lignin        | 10.32           |
| Ash           | 1.22            |

Source: Anindyawati et al., 2012

![Figure 1](image1.png)  
**Figure 1.** Effect of substrate loading on reducing sugar formation at 3.85% (v/v) of enzyme concentration and temperature of 50°C (a), 40°C (b), and 32°C (c). 

**Gambar 1.** Pengaruh konsentrasi substrat terhadap pembentukan gula tereduksi pada konsentrasi enzim 3.85% (v/v) dan suhu 50°C (a), 40°C (b), dan 32°C (c).
Enzymatic hydrolysis of Indonesian oil palm empty fruit bunch……..(Barlianti et al.)

**Effect of enzyme concentration**

The enzyme concentration variations that used in this experiment were 2.574% (v/v), 3.852% (v/v), and 5.148% (v/v). There was slightly increasing of hydrolysis rate at 3.852% (v/v) of enzymes concentration if compared to the hydrolysis rate at 2.574% (v/v) (Figure 4). Application of 5.148% (v/v) enzyme concentration in the experiment did not change hydrolysis rate and reducing sugar concentration at the end of process. This phenomenon might be caused by product inhibition that hinder the enzyme activity to convert cellulose to reducing sugar. It means that 3.852% (v/v) of enzyme concentration was enough to hydrolyze cellulose contained in EFB. The same phenomenon happened on brewer’s spent grain hydrolysis with enzyme dosages around 15-85 FPU (Musatto, 2008). At enzyme dosages of 15-45 FPU, there was significant increasing of sugar production, but at range of 45-85 FPU the final sugar concentration and hydrolysis rate did not change.
Kinetic parameters determination

There are many kinetic models for enzymatic hydrolysis of lignocellulosic materials, some of the models based on Michaelis-Menten kinetics model, the others based on Langmuir isotherm adsorption pattern, and so on. One of kinetic model based on Michaelis-Menten equation is a model that developed by Shen & Agblevor (Zhang et al., 2010). The assumption that used in this model are cellulase enzyme has a single combined effect on hydrolysis reaction of insoluble substrate to produce reducing sugar, the surface and structure of lignocellulosic materials as insoluble substrate is considered homogeneous (Shen & Agblevor, 2011), and in effective production of enzyme-substrate complex that caused by enzyme deactivation reaction (Saura, 2011).

The enzymatic hydrolysis reaction was described that enzyme is adsorbed on the active site of lignocellulosic surface to form enzyme-substrate complex via a reversible reaction. Then enzyme-substrate complex is changed to reducing sugar or glucose and free enzyme. This reaction is showed in Equation 1, while the equations of Shen-Agblevor model were shown in Equation 2 and 3, where \( E_T \) is total enzyme (g/L), \( S_p \) is initial substrate (g/L), \( P \) is product (g/L), \( K_M \) is equilibrium constant (g/L), \( k_{cat} \) is constant of product formation (h\(^{-1}\)), \( k_{de1} \) (h\(^{-1}\)) and \( k_{de2} \) (L/h.g) are first order and second order rate constant of enzyme deactivation. Equation 1 describe hydrolysis reaction of EFB to produce glucose, Equation 2 and 3 illustrated kinetic models with first order and second order of enzyme deactivation reaction.

\[
E + S \rightleftharpoons ES \rightleftharpoons E + P \tag{1}
\]

\[
[P] = [S_0] \times \left[1 - \left(1 - \frac{1 - e^{k_{de1}t}}{1 + e^{k_{de1}t}} \right) \frac{k_{cat}}{k_{de1}} \right] \tag{2}
\]

\[
[P] = [S_0] \times \left[1 - \left(1 + \frac{k_{de1}t}{k_{de2} + k_{de1}t} \right) \frac{k_{cat}}{k_{de1} + k_{de2}} \right] \tag{3}
\]

In this study, to determine kinetic parameters of enzymatic hydrolysis of EFB, the temperature and substrate loading were fixed at the best condition with the enzyme concentration were considered as variable in kinetic models. The value of kinetic parameters was concluded from the best curved fitting of the experimental data with Shen and Agblevor models. The term product (\( P \)) in the equations refers to glucose concentration (main component of reducing sugar in this study), while initial substrate (\( S_p \)) refers to cellulose concentration (main component of EFB). The results were shown in Figure 5.

Figure 5 showed the plot of experimental data versus data values from the first model and the second model (each model refers to equation 2 and equation 3). The experimental datas were closer to the dashed line (refers to model 1 or Equation 2). The kinetic parameter values (\( K_M, k_{cat}, k_{de1} \), and \( k_{de2} \)) were put in Table 2. These models also were applied on steam-explored wheat straw hydrolysis and the selected model was the model with second order of enzyme deactivation reaction (Zhang et al., 2010).

![Figure 5](image-url)
Table 2. Parameter values of EFB hydrolysis based on Shen and Agblevor kinetic model.

| Parameter                          | Symbol | Equation 2 | Equation 3 |
|------------------------------------|--------|------------|------------|
| equilibrium constant               | $K_{d}$ (g/L) | 30.8597    | 27.3084    |
| constant of product formation      | $k_{cat}$ (h$^{-1}$) | 0.0842     | 0.0793     |
| constant of enzyme deactivation    | $k_{de1}$ (h$^{-1}$) | 0.1001     | -          |
|                                    | $k_{de2}$ (L/h.g) | -          | 0.0068     |

Conclusion

Substrate loading, enzyme concentration, and reaction temperature are important factors that affect effectiveness of an enzymatic hydrolysis reaction of lignocellulose materials. The highest sugar concentration was achieved on 20% (w/v) substrate loading and 3.852% (v/v) mixed enzyme of cellulase and β-1,4 glucosidase concentration at 50°C, but the optimal initial substrate concentration with respect to sugar yield was 18%. The kinetics of EF hydrolysis was preferable to follow Shen and Agblevor model with first order of enzyme deactivation reaction. Economic feasibility should be studied to apply the hydrolysis method in commercial scale.

Acknowledgement

This research was funded by Insentif Riset Sistem Inovasi Nasional (SiNas) 2013 Programme, Ministry of Research and Technology, Indonesia. The authors also thanked to Mr. Azni Aulia and Miss Chandra Dea who helped in valuable discussion and technical assistance.

References

Anindyawati T, E Triawahyuni & T Idiyanti (2012). Optimasi proses sakarifikasi tandan kosong kelapa sawit untuk bahan baku bioetanol. Dalam : Prosiding Seminar Nasional XXI “Kimia Dalam Industri dan Lingkungan”. Yogyakarta, 6 Desember 2012.

Baharuddin AS, M Wakisaka, Y Shirai, S Abd-Azis, NA Abdul Rahman & MA Hassan (2009). Co-composting of empty fruit bunches and partially treated palm oil mill effluents in pilot scale. Internat J Agricult Res 4 (2), 69-78.

Banerjee S, S Mudliar, R Sen, B Giri, D Satpute, T Chakrabarti & RA Pandey (2010). Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. Biofuels Bioprod Bioref 4, 77-93.

Bansal P, M Hall, MJ Realff, JH Lee & AS Bommarius (2009). Modelling cellulose kinetics on lignocellulosic substrates. Biotechnol Adv 27, 833-848.

Chen M, L Xia&P Xue (2007). Enzymatic hydrolysis of corncob and ethanol production from cellululosic hydrolysate. Int Biodeter Biodegr 59, 85-89.

Ditjenbun (2014a). Luas areal perkebunan angka estimasi Tahun 2013. Diunduh dari : http://ditjenbun.pertanian.go.id/statis-35-huasareal.html[14 April 2014]

Ditjenbun (2014b). Produksi komoditas tanaman perkebunan angka estimasi Tahun 2013. Diunduh dari : http://ditjenbun.pertanian.go.id/statis-36-produksi.html[14 April 2014].

Kim KC, SW Kim, MJ Kim & SJ Kim (2005). Saccharification of food wastes using cellulolytic and amylolytic enzymes from Trichoderma harzianum FJ1 and its kinetics. Biotechnol Bioprocess Engin 10, 52-59.

Mahamud MR & DJ Gomes (2012). Enzymatic saccharification of sugarcane bagasse by the crude enzyme from indigenous fungi. J Sci Res 4 (1), 227-238.

Musatto SI, G Dragone, M Fernandes, AMF Milagres & IC Roberto (2008). The effect of Agitation speed, enzyme loading, and substrate concentration on enzymatic hydrolysis of cellulose from brewer’s spent grain. Cellulose, 15, 711-721.

Rosgaard L, P Andric, KD Johansen, S Pedersen & AS Meyer (2007). Effects of substrate loading on enzymatic hydrolysis and viscosity of pretreated barley straw. Appl Biochem Biotechnol 143, 27-40.

Saura AS (2011). Modeling and analysis of enzymatic hydrolysis of lignocellulosic substrates. Thesis. KTH Chemical Science and Engineering, Stockholm, Sweden, p. 32-34.
Shen J & AF Agblevor (2011). Optimization of enzyme loading and hydraulic time in hydrolysis of mixture of cotton gin waste and recycled paper sludge for the maximum profit rate. *Biochem Engin J* 41, 241-250.

Styarini D, L Risanto, Y Aristiawan & Y Sudiyani (2012). Comparison of two analytical methods for compositional analysis of lignocellulosic biomass for bioethanol production. *Internat J Environment and Bioenergy* 3(2), 88-97.

Sun Y & J Cheng (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technol* 83, 1-11.

Yao MJ, ZL Wang, ZQ Wu & HS Qi (2011). Evaluating kinetics of enzymatic saccharification of lignocellulose by fractal kinetic analysis. *Biotechnol Bioprocess Engin* 16, 1240-1247.

Yunus R, SF Salleh, N Abdullah & DRA Biak (2010). Effect of ultrasonic pretreatment on low temperature acid hydrolysis of oil palm empty fruit bunch. *Biores Technol* 101(24), 9792-9796.

Zhang Y, JL Xu, HJ Xu, ZH Yuan & Y Guo (2010). Cellulase deactivation based modeling of enzymatic hydrolysis of steam-exploded wheat straw. *Biores Technol* 101, 261-826.