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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

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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 alkalindi ikuti dengan hidrolisis TKKS secara enzimatik menggunakan kombinasi enzim selulase 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 selulase dan β-1,4 glukosidase sebesar 3,85% (v/v), dan suhu 50°C. Perbandingan antara selulase 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 reakside 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, selulase, 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 consist 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 enzimatically using cellulase enzyme, a complex enzyme that consists of exocellulase or exobiohydrolase, endocellulase or endo-β-1,4-glucanase and β-1,4-glucosidase orcellobiase. Enzymatic hydrolysis process still hampered by slow reaction rate that

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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 lignocelluloses 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/cm^2 for 30 minutes. As much as50 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 oneand 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 Cu_2O 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 within creasing 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 oil palm EFB.

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

Source :Anindyawati *et al*., 2012

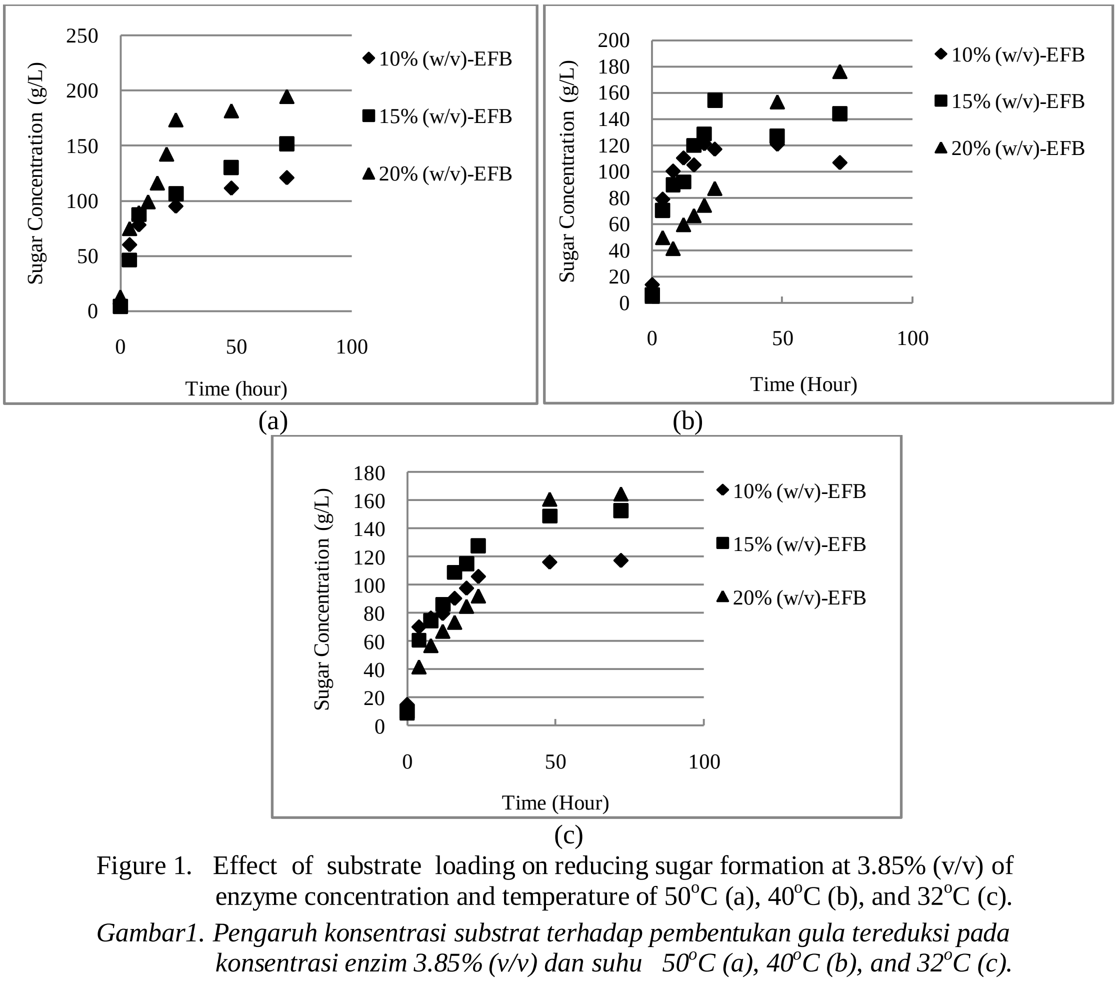

Figure 1. Effect of substrate loading on reducing sugar formation at 3.85% (v/v) of enzyme concentration and temperature of 50°C (a), 40°C (b), and 32°C (c).

Gambar1. Pengaruh konsentrasi substrat terhadap pembentukan gula tereduksi pada konsentrasi enzim 3.85% (v/v) dan suhu 50°C (a), 40°C (b), and 32°C (c).

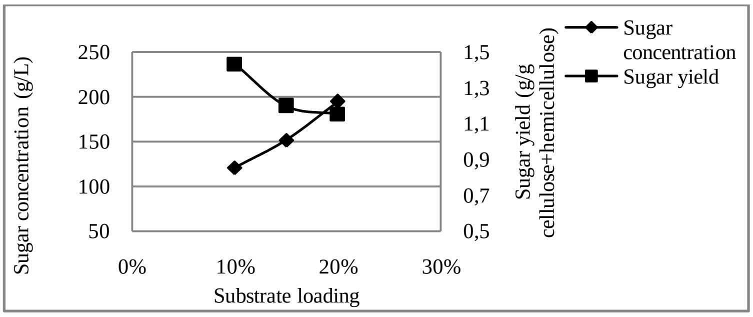

Figure 2. Sugar concentration and yield produced at different substrate loading at 50°C and 3.85% (v/v) of enzyme concentration.

Gambar 2. Konsentrasi dan hasil gula tereduksi yang diperoleh pada berbagai konsentrasi substrat pada suhu 50°C dan konsentrasi enzim 3.85% (v/v).

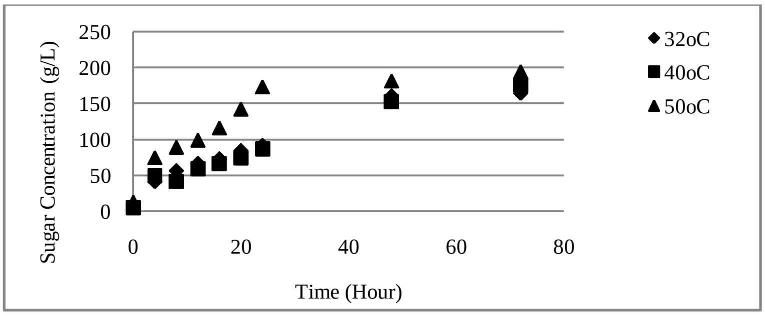

Figure 3. Effect of hydrolysis temperature on reducing sugar formation at 20% (w/v) substrate loading and 3.85% (v/v) of enzyme concentration.

Gambar 3. Pengaruh suhu hidrolisis terhadap pembentukan gula tereduksi pada konsentrasi substrat 20% (w/v) dan konsentrasi enzim 3.85% (v/v).

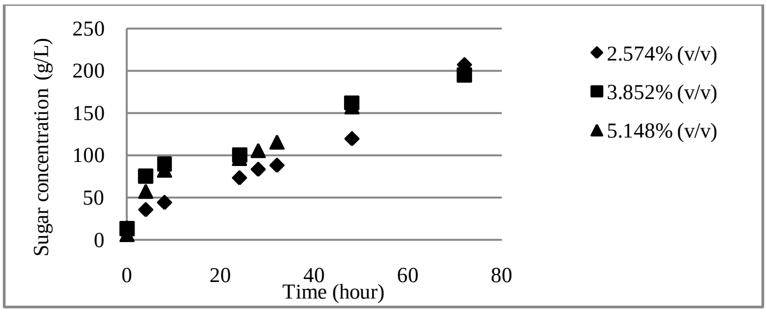

Figure 4. Effect of enzyme concentration on reducing sugar formation at 50°C and 20% (w/v) substrate concentration.

Gambar 4. Pengaruh konsentrasi enzim pada pembentukan gula tereduksi pada suhu 50°C dan konsentrasi substrat 20% (w/v).

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 modelbased 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_o 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.

$$E + S \leftrightarrows ES \xrightarrow{k_{cat}} E + P \qquad (1)$$

$$[P] = [So] \times \left[1 - \left(1 - \frac{1 - exp(k_{de1}t)}{1 + \frac{K_M}{E_T}}\right)^{\frac{k_{cat}}{k_{de1}}}\right] \qquad (2)$$

$$[P] = [So] \times \left[1 - \left(1 + \frac{K_M E_T}{K_M + E_T} k_{de2} t\right)^{\frac{-k_{cat}}{K_m k_{de2}}}\right] \qquad (3)$$

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 wasconcluded 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_o) refers to cellulose concentrate-ion (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-exploded wheat straw hydrolysis and the selected model was the model with second order of enzyme deactivation reaction (Zhang *et al*., 2010).

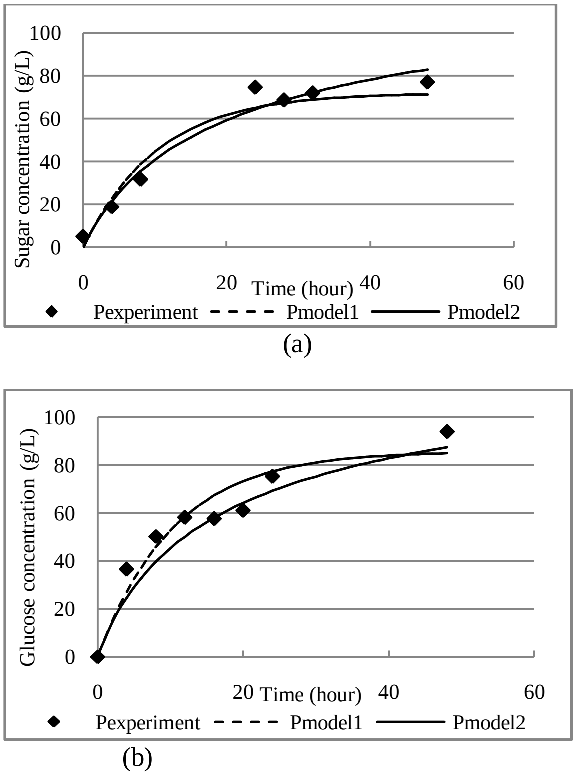

Figure 5. Glucose concentration prediction versus experimental data at 50^{o}C, 20% (w/v) of substrate loading, and 2.574% (v/v) of enzyme concentration (a) and 3.852% (v/v) of enzyme loading (b).

Gambar 5.Prediksi konsentrasi glukosa versus data percobaan pada suhu 50^{o}C, konsentrasi substrat 20% (w/v), dan konsentrasi enzim 2.574% (v/v) (a) dan konsentrasi enzim 3.852% (v/v) (b).

Table 2. Parameter values of EFB hydrolysis based on Shen and Agblevor kinetic model.

Table 2.Nilai parameter hydrolysis TKKS berdasarkan model kinetika Shen dan Agblevor.

Parameter	Symbol	Equation 2	Equation 3
equilibrium constant	K_M (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 glucosidaseconcentration at 50°C, but the optimal initial substrate concentration with respect to sugar yield was 18%. The kinetics of EFB 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.

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