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Kinetic Study of Oil Palm Empty Fruit Bunch Enzymatic Hydrolysis

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Abstract

As lignocellulosic biomass, Oil Palm Empty Fruit Bunch (OPEFB) can be used as the source of xylose that can be further utilized as the raw material for xylitol production. The processing of OPEFB to xylose comprises of pretreatment and hydrolysis that can be performed enzymatically. This process offers the advantages of moderate operation conditions and more environmentally friendly. This article describes the kinetic study of enzymatic hydrolysis process of OPEFB for producing xylose using self-prepared and commercial xylanase enzymes. Despite the possible mass transfer limitation, the Michaelis Menten kinetics was hypothesized. The results indicated that the reaction at pH 5 and 60°C followed the Michaelis Menten kinetics, with V_m of 0.84 g/L-h and K_m of 48.5 g/L for the commercial enzyme, and V_m of 0,38 g/L-h and K_m of 0,37 g/L for the self-prepared enzyme. The reaction is affected by temperature, with E_a of 8.6 kcal/gmol. The performance of self-prepared xylanase enzyme was not yet as good as the commercial enzyme, Cellic Htec 2.

Keywords: *enzymatic hydrolysis; kinetics parameter; OPEFB; xylanase; xylose*

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INTRODUCTION

Oil palm is one of the important industrial plants in Indonesia. Recorded until the end of 2015, the estimated area of productive Indonesia's oil palm plantations is ± 10.9 million hectares (Ditjenbun, 2014), while the annual Crude Palm Oil (CPO) production reached ± 32.5 million tonnes. About 23% of Fresh Fruit Bunch ends up as the Oil Palm Empty Fruit Bunch (OPEFB), which is now commonly used only as organic fertilizer or reused in the oil palm plantations as the mulch. However, not all of OPEFB can be used as organic fertilizer, so it will surely be accumulated and raise an environmental problem.

Lignocellulosic biomass composed of polysaccharides, such as cellulose and hemicellulose as well as lignin. In the concept of biorefinery, lignocellulosic biomass can be treated in the following step. First is pretreatment for releasing cellulose and hemicellulose, followed by hydrolysis to depolymerize polysaccharide to its free sugar residual. Finally, fermentation is conducted to convert free sugars to more valuable product (Dutta and Chakraborty, 2015).

Xylose is one of those free sugars stated earlier. It can be used to produce xylitol, a polyol with almost the same sweetness as sucrose but much safer for

tooth and for diabetic patients. One of lignocellulosic biomass that has been considered as a source of xylose is the OPEFB. According to (Mardawati, 2015) the hemicellulose content in OPEFB of 23.3 ± 0.52%, 84% of which comprises of xylose. That value is promising enough for OPEFB to be utilized as a source of xylose. In order to get xylose from OPEFB, it has to be depolymerized by hydrolysis either via chemical process using aqueous strong acid or base, or via enzymatic hydrolysis. The latter process is milder in process condition, conducted at pH around 5 and in a mild temperature of 45-50°C (Hsu *et al.*, 2010) or 60°C (Mardawati *et al.*, 2014).

Xylan is part of hemicellulose that is composed of xylopyranose unit, connected by 1,4-β glucosidic linkage. It can only be completely hydrolyzed by using a complex xylanase enzyme, comprises of endo-1,4-β-D-xylanase, β–xylosidase, α–arabinosidase, and α – glucuronosidase activities. The endo-1,4-β-D-xylanase (EC3.2.1.8) hydrolyse the backbone of xylan to produce short chain xylooligosaccharide. The β–xylosidase, hydrolyses short chain xylooligosaccharides to produce monomer, xylose. While α–arabinosidase responsible to produce arabinose from arabinoxylan, arabinogalactan or arabinan. OPEFB consists of arabinoxylan instead of arabinogalactan or arabinan. Then α–glucuronosidase responsible to produce D-glucoronic acid. but it is usually found in hardwood (Marais, 2008).

Xylan degrading organisms have been found to produce multiple enzyme (Marais, 2008). They can be bacteria, fungi or yeast. However, the produced xylanase activity depends on the composition of the growth media. For example, crude xylanase produced from microbial cultivation on OPEFB may also contains glucose and laccase activity (Mardawati, 2015).

Xylose yielded can be used to produce xylitol by means of fermentation process. In the view of production of xylitol from OPEFB hydrolysate via fermentation, the ratio of xylose to glucose in the hydrolysate used as substrate for fermentation is an important parameter that affecting the selectivity of xylitol production to biomass or ethanol. It is very important to have the appropiate xylose to glucose ratio in the OPEFB hydrolysate in order to gain high xylitol selectivity (Mardawati *et al.*, 2014). Further the higher the yield of xylose from the hydrolysis of OPEFB gives higher xylose concentration in the hydrolysate, providing higher xylose concentration for fermentation. This will facilitate high xylitol concentration obtained in the fermentation broth.

In order to control the hydrolysis reaction, kinetic study of the reaction is important. It is the aim of this research, to study the kinetics of OPEFB enzymatic hydrolysis reaction. In general, the effect of temperature on enzyme kinetics will be discussed and the performance of commercial and self-prepared enzyme will be compared.

EXPERIMENTAL DETAIL

Raw Material

OPEFB used in this research was kindly provided by oil palm plantation in West Sumatra, PT Incasi Raya. It went through some preparation process: cleaned, sun dried for a few days and then followed by oven dried at 60°C for 24 hour until the moisture content was 2%. Then the OPEFB was milled in a disc mill and sieved to a particle size -60+80 mesh.

The self-prepared xylanase used was prepared from *Trichoderma viride ITB CC L67 cultivation* using OPEFB as substrate following (Mardawati, 2015). The activity of the self-prepared xylanase was reported to be 5180.6 U/g-substrate (Mardawati *et al.*, 2014). In this research, the performance of the self produced xylanase was compared with commercial enzyme, Cellic Htec2 (Novozyme). Cellic HTec2 is a mix between endoxylanase and cellulase that can hydrolyze hemicellulose into its constituent monomers. Cellic Htec2 had been tested for its activity of 38,443.95 U/mL.

Chemicals like citric acid and sodium citrate for buffer was technical grade. Xylose and glucose was from purchased from Sigma-Aldrich.

Experimental Methods

The experiments were conducted to find the kinetic parameters of OPEFB enzymatic hydrolysis reactions that are V_m, K_m and E_a.

OPEFB used were 0,1, 0.5, 1, 1.5, 3, and 5 g/100 mL solution for the self-prepared enzyme, and 1 and 5 g/100 mL solution for the commercial enzyme. Since the two enzymes have different activity, it was preset that enzyme used must have the same total activity. The enzyme used was 25 mL for self-prepared enzyme and 1.5 mL for Cellic Htec2. The citrate buffer solution, 0.05 M pH 5.0, was added up until working volume of 100 mL. Experiments were performed in 250 mL erlenmeyers.

Prior to the addition of enzyme solution, the mixture of OPEFB and buffer were autoclaved at 121°C for 15 minutes. The hydrolysis reactions were conducted at the preset temperature, 45, 50, or 60°C, and at 150 rpm aseptically in a shaking incubator (Daihan Labtech Co.Ltd). Samples were taken aseptically every 30 minutes until the duration of experiments, 150 minutes. Samples were centrifuged at 6000 rpm for 15 minutes then filtered by 0.22 mm micro filter before further sugar concentration analysis of the hydrolysate.

The sugar composition of hydrolysate was analysed by HPLC (Alliance HPLC System, Waters) following the method of Sluiter et al. (2008). The OPEFB composition analysis was conducted following TAPPI Standards in Balai Besar Pulp dan Kertas, Bandung, West Java. The method used for lignin (T 13 os-54) and pentosans (T 19 m–50). Overall experiments were run in duplo.

Kinetic Parameter Estimation

The kinetics of an enzyme-catalyzed reaction is often referred to Michaelis Menten kinetic (Shuler and Kargi, 2002). The Michaelis Menten kinetic equation is written as follows,

$$v = \frac{d[P]}{dt} = \frac{v_m S}{K'_m + S} \quad (1)$$

The extent of enzymatic hydrolysis reaction was monitored by measuring the xylose concentration in hydrolysate. The results from HPLC were then processed to find the V_m and K_m of the self-prepared xylanase and commercial one following plotting used of Lineweaver-Burk, Eadie-Hofstee or Hanes-Woolf. (Shuler and Kargi, 2002).

The rate of enzyme-catalyzed reaction increases with temerature up to a certain limit (Shuler and Kargi, 2002). This temperature limit presents an optimal temperature and the energy to reach that limit is the activation energy, E_a. The activation energy is the minimum energy required to reach transition state of reactant (substrate) conversion to product. The rate varies according to Arrhenius equation is written as,

$$k = Ae^{\frac{-E_a}{RT}} \quad (2)$$

This article determined the Arrhenius constant of the enzymatic reaction only for reaction using commercial enzyme.

RESULTS AND DISCUSSION

As mentioned before, there are two kinds of experiments: one with self-prepared enzyme and the other one with commercial enzyme. The kinetics parameters were determined from each set of experiments

Kinetics Parameters Estimation

HPLC analysis data using self-prepared enzyme were processed to obtain time profile of the xylose as the product of the enzyme hydrolysis proses is presented in Figure 1.

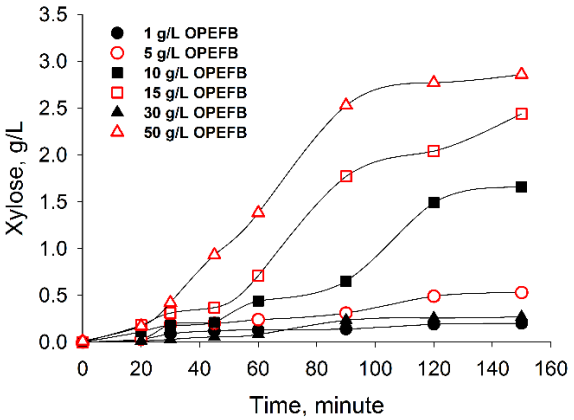

Figure 1. Xylose concentration profile through time using self-prepared enzyme at 60°C

Figure 1 shows that xylose concentration increase with time. The lines showed different slope for the steepest is for 50 g/L OPEFB and the slightest is for 1 g/L OPEFB. This is due to the different amount of substrate in every condition. The more substrate means that it will produce more product. While for commercial enzyme are shown in Figure 2 to 4.

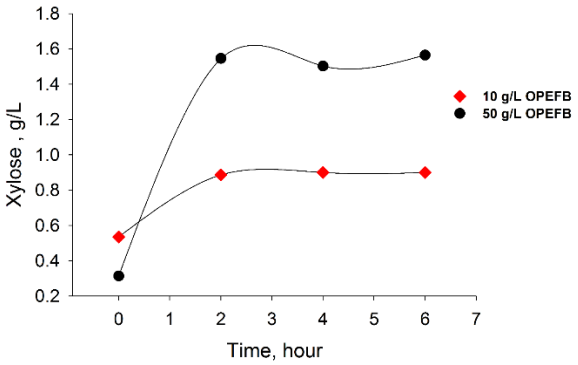

Figure 2. Xylose concentration profile through time using commercial enzyme at 45°C

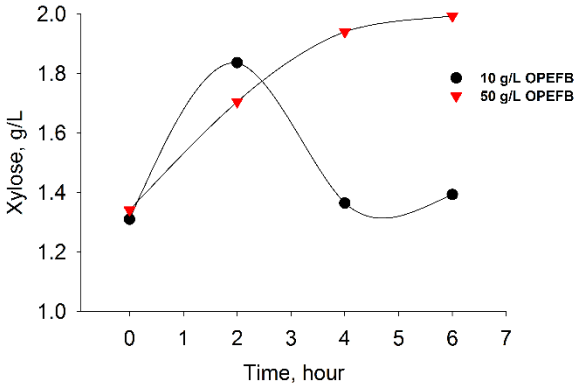

Figure 3. Xylose concentration profile through time using commercial enzyme at 50°C

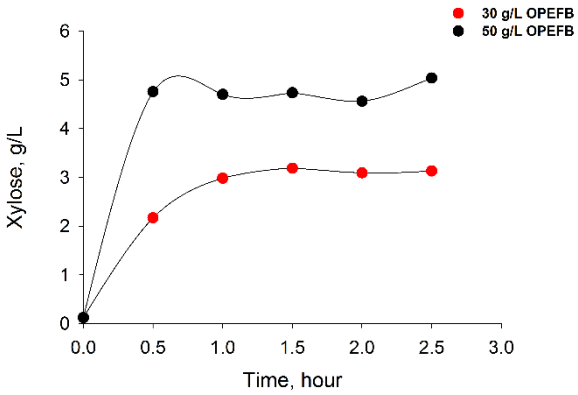

Figure 4. Xylose concentration profile through time using commercial enzyme at 60°C

Figure 2 to 4 for commercial enzyme showed that xylose as product also increase with time. In the first two hours the slope is steep and then get slighter. Temperature plays important role, as can be seen in

Figure 4, the slope is steep in the first half-hour. This showed that Cellic HTec2 works faster at 60°C to produce xylose.

In order to obtain the kinetic parameters, a linearization method was performed. The method used was Lineweaver-Burk method, and the graph are shown in Figure 5 for the self-prepared enzyme and Figure 6 for the commercial xylanase one. Both figures showed that the observed data can be best fitted as linear, with R-square values of 0.988, 0.997 and 0.804 for the enzymatic hydrolysis process OPEFB using commercial xylanase enzyme at 45, 50, and 60°C; and with R-square value of 0.865 for the enzymatic hydrolysis process of OPEFB using the self-prepared xylanase enzyme at 60°C.

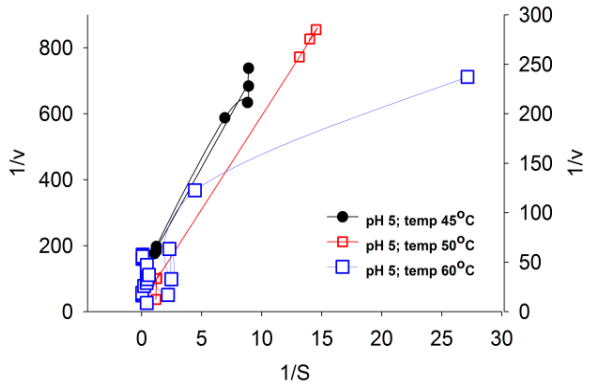

Figure 5. Lineweaver-Burk linearization for enzymatic hydrolysis of OPEFB using commercial enzyme at various temperatures. Data taken at pH 5 and 45°C is plotted using the left axes

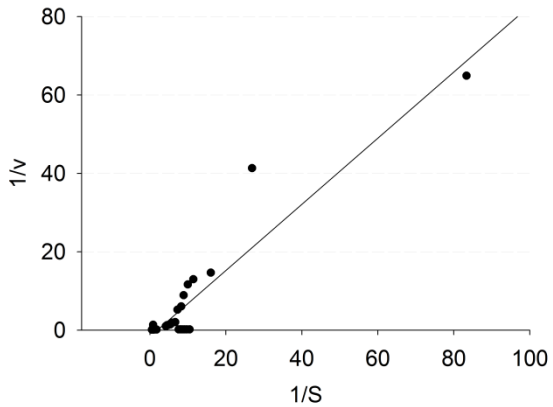

Figure 6. Lineweaver-Burk linearization for enzymatic hydrolsyis of OPEFB using self-prepared xylanase enzyme at 60°C, pH 5

Followingly, the estimated kinetics parameters of the OPEFB enzymatic hydrolysis reactions are presented in Table 1.

Table 1 shows thaat at the same temperature, 60°C, the measured v_m for the self-prepared enzyme is about 65% of the commercial one. v_m represents the maximum rate attainable, the rate at which the total enzyme concentration is present as the enzyme–substrate complex (Chang, 2005). This indicates that the self-prepared xylanase enzyme is not yet as good as the commercial one. At the same temperature, lower value of K_m was also observed for the self-prepared enzyme, 0.37 g/L, compared to the one of commercial xylanase enzyme, 13.7 g/L. This shows that the self-prepared enzyme has higher affinity for OPEFB compared to the commercial one. Overall, although the self-prepared xylanse enzyme is not as fast as the commercial one, it is more suitable for OPEFB.

Table 1. Kinetics parameters for the enzymatic hydrolysis of OPEFB using self-prepared and commercial xylanase enzymes

Enzyme used	Kinetic Parameters					
	v_m, g/L-hour			Km, g/L		
	45°C	50°C	60°C	45°C	50°C	60°C
Commercial xylanase enzyme	0.27	0.84	0.59	5.9	48.5	13.7
Self-prepared xylanase enzyme	-	-	0.38	-	-	0.37

For comparison, Table 2 below presented literature study on kinetic parameters of purified xylanase used in hydrolysing lignocellulolitic biomass. Along with the kinetics parameters, it also describes the types of microorganism used in producing the enzyme, types of growth media applied, and the types of biomass as the lignocellulosic materials used in the hydrolysis experiments for estimating the kinetics parameters. The biomass used is mainly xylan either from synthetic or natural sources since xylan is the substrate for xylanase.

Table 2. Kinetic parameters of purified xylanase in lignocellulosic hydrolysis

Microorganism used for enzyme production	Growth Media	Biomass	K_m (g/L)	Reff
Thielaviopsis basicola MTCC 1467	Rice straw	xylan	1.447 ±0.22	(Goluguri *et al.*, 2016)
Caldicoprobacter algeriensis sp. novell, strain TH7C1T	Birchwood- and oats spelt-xylan	Oat-spelt xylan	1.33	(Amel *et al.*, 2016)
Bacillus pumilus	Synthetic media contains xylan	xylan	2.3	(Kapilan and Arasaratnam, 2011)
Penicillium rolfsii c3-2 IBRL	Synthetic media contain birchwood xylan	Oil palm trunk	5.73	(Lee *et al.*, 2015)
Bacillus subtilis-BS05	Sugarcane bagasse	Birchwood xylan	1.15	(Irfan *et al.*, 2013)
Aspergillus niger	Birchwood xylan	Birchwood xylan	2.94	(Savanth and Patel, 2014)
T. viridae ITBCC L67	OPEFB	OPEFB	0.37	This study

As seen on Table 2, our crude xylanase enzyme has K_m much lower that other reported values. K_m is an intrinsic parameter, and depends on pH or temperature (Shuler and Kargi, 2002). Besides it depend also on xylan source and the type of xylan or hemicellulose it is used upon. The data thus shows that our self-prepared enzyme has a big affinity for xylan of OPEFB. It is a good point for us to believe that this self-prepared enzyme can be upgraded for it's better performance since it is still a crude enzyme. As can also be seen on Table 2, The highest K_m value of 5.73 g/L was obtained by using synthetic xylan as growth media of fungus used. The K_m value of commercial enzyme used in this experiments was estimated to be 5.9-48.5 g/L, higher than the average reported data in the literature.

Effects of Temperature

The effects of temperature were studied using commercial enzyme only. The results are shown as a plot of ln v vs 1/T in Figure 7 below,

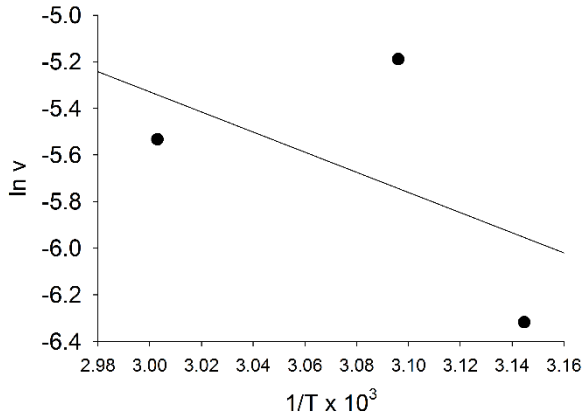

Figure 7. Arrhenius plot for commercial enzyme

The graph gave a regression equation to calculate the Ea value of 8.6 kcal/gmol. This value is fair enough since mostly enzyme has Ea of 11 kcal/gmol (Shuler and Kargi, 2002).

CONCLUSION

The K_m value for commercial enzyme is 0.27, 0.84 and 0.58 g/L at 45, 50, and 60°C respectively. The v_m value is 5.9, 48.5, and 13.7 g/L-hr at 45, 50, and 60°C respectively. While for the self-prepared enzyme the K_m and v_m value at 60°C is 0.37 g/L and 0.38 g/L-hr respectively. Although the self-prepared enzyme has a lower v_m value, it has a higher affinity to OPEFB considering it;s higher K_m value. The self-prepared enzyme has a potentially capability to increase the value since it is still a crude enzyme.

ACKNOWLEDGMENTS

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NOTATION

K_m : Michaelis-Menten constant, g/L
vm : Maximum forward velocity of a reaction, g/L-hr
v : Rate of product formation or substrate consumption, g/L-hr
S : Substrate concentration, g/L

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