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Optimization and Kinetic Modelling of The Enzymatic Hydrolysis of Oil Palm Petioles

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Abstract

Oil palm petiole is the solid waste of the crude palm oil industry. It contains about 35% cellulose, 18% hemicellulose and 22-25% lignin. During hydrolysis lingo celllulosic, cellulose and hemicellulose are gradually degraded into fermentable sugars, such as glucose and xylose. Enzymatic hydrolysis of oil palm petioleby xylanase could be an effective biotechnological process, since it can be performed at ambient temperature and pressure. Further glucose and xylose can be used as raw material for the production of a wide variety of chemicals such as xylitol and bioethanol. The aim of this study wasto examine the optimum conditions needed for the enzymatic hydrolysis of oil palm petioles, particularly temperature and pH. A surface Response Method Methodologies (RSM) by central composite design (CCD) was employed to obtain the optimum xylose concentration. The dynamics of enzymatic hydrolysis process was modelled using the Michaelis Menten kinetic model with kinetic parameters obtained from experimental data. The results of this study lead to an enhanced process of the enzymatic hydrolysis of oil palm petiole, whichwas shown to follow the Michaelis Menten kinetic model and the kinetic parameters including Km and Vm were obtained, they were 6.433 g/L and V_m = 0.042 g/L/min. The optimum hydrolysis condition were observed to be at temperature 50°C and pH 4.8.

Keywords: enzymatic hydrolysis; glucose; kinetic modelling; oil palm petioles; xylose.

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1. Introduction

Palm oil is a potential agricultural product in Indonesia, particularly in improving the economy of the country. Indonesia produced 17.5 million tons crude palm oil (CPO) in 2008 which increased up to24.4 million tons in 2013 with a productivity of 3600 kg/ha [1]. The world's high demand for CPO is utilized in the food industry for cooking oil, margarine and emulsifiers, cosmetics, detergents, as well as for the energy industry as a raw material for biodiesel and bioethanol production.

Along with the increase of oil palm cultivation and the processing of palm oil, the CPO industry also produces various wastes, respectively the stem, stem leaf, husk,

shell, empty fruit bunches and oil palm petioles (OPP) [2]. The largest solid waste produced during cultivation of palm oil is the petioles (OPP), it was 30% of palm oil plant. Processing of waste oil palm biomass is absolutely necessary in addition to produce more valuable products, as well as to prevent negative socio-environmental impacts of the palm oil industry [3]. Currently, OPP are mostly returned to the farm as compost. As ligno celulose material, OPP consists of cellulose as the main fraction, hemicellulose and lignin [4].

This biomass waste has a high potential to be used as raw material for various chemical processes following the concept of biorefinery. For example cellulosic material could be used as the feedstock of bioethanol, biofuel, pulp mills and others. Furthermore the hemicellulose could also be used to get a variety of valuable products. In the utilization of hemicellulose, it must first be hydrolyzed into its components, that are the 6 carbon ataom sugar (mannose and galactose) and 5 carbon atom sugar (xylose and arabinose). This process could be performed chemically, at high pressure and temperature using acid or alkaline as the catalyst or at ambient condition using biological xylanolytic enzim as the catalyst [5]. Xylose is the largest constituent monomers in hemicellulose. Xylose hydrolysate of OPP can be exploited further became the sweetener of xylitol [6].

The factors that affect the enzymatic hydrolysis of lignocellulosic material including OPP are substrates, enzyme activity, and reaction conditions (temperature, pH, as well as other parameters) [7]. To improve the yield and rate of the enzymatic hydrolysis of OPP, it is necessary to know the optimal conditions for enzymatic hydolysis particularly temperature, pH and substrate concentration. Different enzymes and substrate have different optimum temperature and pH values. In hydrolysis process, if temperature increases, initial rate of reaction will increase since increased kinetic energy. Yet, the effect of bond breaking will become bigger up to the condition will cause the active site to change shape and the rate of reaction will begin to decrease. Furthermore, the increasing of substrate concentration will affect to increases the rate of reaction. This is because more substrate will be reacted with enzyme molecules, it will generate more product resulted. However, after a certain concentration, the increasing of substrate concentration will have no or constant effect on the rate of reaction, because of saturated enzyme and limiting factor [8].

The objective of this research to optimize the hydrolysis process of OPP using commercial xylanase. The research included determining the optimum conditions in the enzymatic hydrolysis of OPP respectively pH and temperature. Furthermore to explore the influence of substrate concentration on the enzymatic hydrolysis process, the dynamics and kinetics of the hydrolysis process.

Table 1: The value of each variable for Optimization of Enzymatic Hydrolysis with CCD.

Factors/variables	(-α)	(-)	(0)	(+)	(+α)
pH	3.5	4	5	6	6.5
Temperatur, (°C)	36	40	50	60	65

*(-α and +α: axial point), (-: minimal point), (0: central point), (+: maximal point)

2. Material and Methods

2.1. Preparation of OPP

OPP collected from the Cikasungka palm oil mill, Bogor, Indonesia, was sun dried until total solid ±80%, cleaned, oven dried at 105°C overnight ground and sieved. OPP of, max. 60 mesh was used. OPP composition has been previously determined by the standard method of TAPPI [3].

2.2. Enzymatic Hydrolysis Process Optimization

A300 mL shake flask containing a 100 mL working volume consisting of 3% substrate or 3 g OPP in 25 mL acetate buffer 0.05M pH 5 was autoclaved at 121°C, 10 min and cooled at room temperature, and then added 75 mL of 1% commercial xylanase enzyme (Cellic Htec manufactured by Novozyme) at 150 rpm for 72 h in incubator shaker. A Central Composite Design (CCD) with two factors, five levels, 2 replicates at factorial point, 2 replicates at star point were usedand 5 replicates at the center point. The temperature was varied in the range of 40-60°C with 50°C as center point, whereas the pH was varied between 4-6 with 5°C as center point. The center point was set up at 50◦C and pH 5. The latter experiments were performed for 150 min. Table 1 shows the factors and levels of experiment.

2.3. Enzymatic hydrolysis kinetics

The hydrolysate was obtained by separating the solid contain OPP waste from the mixture by vacuum and centrifugation. The hydrolysate was then analyzed for sugars in terms of xylose. The enzymatic hydrolysis was carried out in an incubator shaker (type IKA®KS 4000 i Control) at 150 rpm. The kinetic were determined using various concentrations of OPP respectively: 5, 7.5, 10, 12.5, and 15% (w/v) or 50, 75, 100, 125 and 150 g/L. Hydrolysis was conducted at optimum temperature and pH obtained previously during 150 min hydolysis time and samples were taken periodcally every 30 minutes.

2.4. Analysis of sugars

Xylose was measured by using the method of Mardawati et.al [3] HPLC using HPX-87H Biorad column and Refractive Index Detector (RID) respectively: mobile phase: 0.005 M sulfuric acid, flow rate: 0.6 mL/minute, column temperature: 60 °C, and detector temperature: 40°C [3].

3. Result and Discussion

3.1. Enzymatic Hydrolysis Process Optimization

The commercial xylanase enzymes CellicHtec (Novozyme) was evaluated in this study. The xylanase activity (U/mL) yielded from the evaluation of this enzymes was measured of 68,490.65 U/mL using Bailey Methods. In order to optimize the enzymatic hydrolysis process, two operation conditions were optimized: temperature and pH. Sugars concentration in term of xylose as product of hydrolysis were analysed by HPLC in 2 duplicate. The objective of this research was to produced maximize xylose synthesis. Table 2 showed factors and results of the experiment.

Table 2 shown that the maximum xylose concentration (1.923 g/mL) was obtained at pH 5 and 50°C then the hydrolysis yield in terms of xylose is 40.6%. Therefore, the theoretical maximum xylose yields calculated from the initial substrate about 4.752 g/L. The lowest hydrolysis yield was obtained with pH 6 and temperature 40°C, that is 5.1% which is only 0.242 g/L xylose released from substrate of OPP. It was caused alkali condition and low temperature not enough temperature to broken the fiber of wall substrate.

The significance of each variable in the CCD experiment were calculated and response surface graphs were generated using Design Expert 7.0 statistical software, the anova analysis for response xylose concentration was shown in Table 3. The accuracy and general ability of the kuadratic model could be evaluated by the determination coefficient (R^2 = 89%). The obtained experimental data was proceesed using multiple regression analysis and the data were fit to quadratic model. The fitted equations for enzymatic hydrolysis for each responses were presented succeedingly in Equations 1 where *Y* represent the response of xylose concentration.

The Student's *t*-distribution and the corresponding *P*-value, along with the parameters was shown in Table 3. The table shown that model of the hydrolysis process is significant because the P values < 0.00001. It means that the model valid to describe the experiment result.

The *P*-values are used as a tool to check the significance of each coefficient, which will help to explain the pattern of mutual interactions between the best variables. The

Table 2: Factors level sand results of the enzymatic hydrolysis optimization.

Run	Factor 1 levels	Factor 2 levels	Response	
	pH	Temperature (°C)	xylose concentration (g/L)	STD (%)
1	5	36	0.382	0.1
2	5	50	1.635	1
3	6.2	50	0.368	5
4	5	50	1.712	4
5	4	40	0.426	2.5
6	5	50	1.923	5
7	5	50	1.224	3
8	6	40	0.242	0.1
9	5	50	1.670	3.5
10	5	65	0.428	2
11	4	60	1.275	5
12	6	60	0.374	5
13	3.5	50	0.974	6
14	5	36	0.351	1
15	6.2	50	0.404	1
16	4	40	0.5032	2
17	6	40	0.295	1
18	5	65	0.452	2
19	4	60	1.321	9
20	6	60	0.312	2
21	3.5	50	0.298	1

parameter coefficient and the corresponding *P*-value suggested that, pH and temperature do has significant effect on xylose concentration as hydrolysis product (P values <0.05). Checking the suitability of the model can also be done using a normal probability plot of the residuals and residual plots against the prediction of response. Normal probability plot of the residuals is deemed to comply if the residual value to be around a straight line. Meanwhile, in response to the prediction residual plot is deemed to comply if the residual value scattered randomly. This shows that the variance of observation is constant for all values of responses. Figure 1a is a normal probability plot of the residuals and residuals shows that in general are in a straight line, which means errors are normally distributed. While Figure 1b shows that the residuals do not show a specific pattern (random).

$$\text{The concentration of xylose (Y)} = -30,84 + 6.49pH + 0,66T - 0.01pH * T - 0,58pH\text{^}2 - 0.005T\text{^}2 \quad (1)$$

Table 3: Analysis of variance of the effect of T° and pH on xylose concentration.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	5.983	5	1.197	25.250	< 0.0001
A-pH	0.037	1	0.037	0.778	0.3916
B-Temperatur	3.363	1	3.634	76.675	< 0.0001
AB	0.29	1	0.287	6.061	0.0264
A^2	3.14	1	3.135	66.160	< 0.0001
B^2	3.49	1	3.486	73.545	< 0.0001
Residual	0.71	15	0.047		

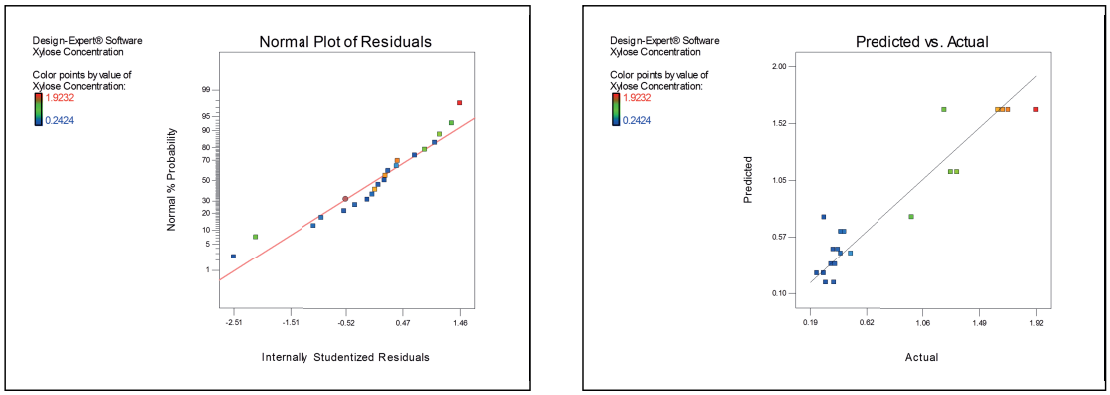

Figure 1: (a,b). The normal probability curve for the response and Residual concentrations of xylose and predictive response to the concentration of xylose.

The effect of pH and temperature on the concentration of xylose hydrolysis can be seen from the three-dimensional profile curve and the curve contour. The sample countour and 3D response surfaces plots were employed to illustrate the interaction of temperature and pH and their effects onenzymatic hydrolysis result (Figure 2).

The optimum condition for enzymatic hydrolysis was presented Figure 2. The optimum temperature for hydrolysis of OPP using commercial xylanase was obtained to be at temperature 50°C and pH 4.8 with the xylose released 1.67 g/L or similiarly with 35% of hydrolysis yield. If compare with hydrolysis of EFB using commercial xylanase (Accelerase XY produced by Genecor), the results showed that temperature and pH was obtained at condition 60 °C, pH 5. This proved that enzyme has high tolerance in high temperature condition [2].

3.2. Enzymatic hydrolysis kinetics

Kinetics of enzymatic hydrolysis experiments using commercial xylanase was conducted with some variation of the substrate concentration of OPP. The variation of the substrate concentration (g / L) was 50-150 g/L or 50, 75, 100, 125 and 150 g/L with the ratio of the buffer to enzyme was at 75% to 25%. The hydrolysis process was carried

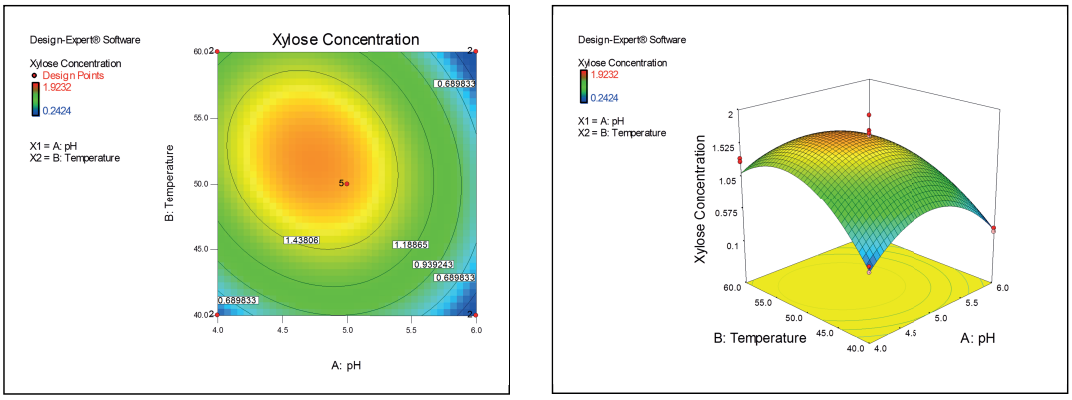

Figure 2: Countour and 3D graph of the effect temperature and pH on the enzymatic hydrolysis of OPP for xylose concentration reponse.

out for 150 minutes and samples were taken periodecally during the hydrolysis, that are 0, 15, 30, 45, 60.90, 120 and 150 minutes. Figure 3 is the curves of the concentration of xylose at varous as a function of substrate concentrations period of observation.

The effect of OPP concentration on hydrolysis rate was shown in Figure 4. The rate of enzymatic hydrolysis was calculated from xylose concentration at 30-90 min, in which the initial rate was assumed to be constant and OPP concentration was converted in xylan. The experiment data shows that the rate of enzymatic hydrolysis increased along with the increase ininitial substrate concentrations. This is consistent with the enzyme kinetic modelling was proposed by Michellis Menten was modelled enzymatic reaction rate as follows [8]:

$$V = V_m[S]/(K_m + [S]) \quad (2)$$

Figure 3 shows that resulted xylose concentration is significantly correlated with hydrolysis time. In general, the longer the hydrolysis resulted in the higher xylose concentration. Maximum xylose concentration was achieved at the end of observation,150 min on 15% or 150 g/L OPP substrate.

Kinetics parameter was estimated via the linearization of Michaellis Menten model, following Lineweaver-Burk plot method as is shown in Figure 4:

Accordingly Fig. 4, the apparent V_{max} and K_m values for the enzymatic hydrolysis were calculated to be K_m = 6.433 g/L and V_m= 0.042 g xylan/L/min with R^2 = 98%. This shows that the process could be well modelled following Michaelis Menten model after 40 min reaction running. K_m demonstrated high affinity of the substrate is low. The smaller the value of Km higher affinity for the substrate, so that the lower the concentration of substrate needed to achieve maximum catalytic reaction rate (Vm).

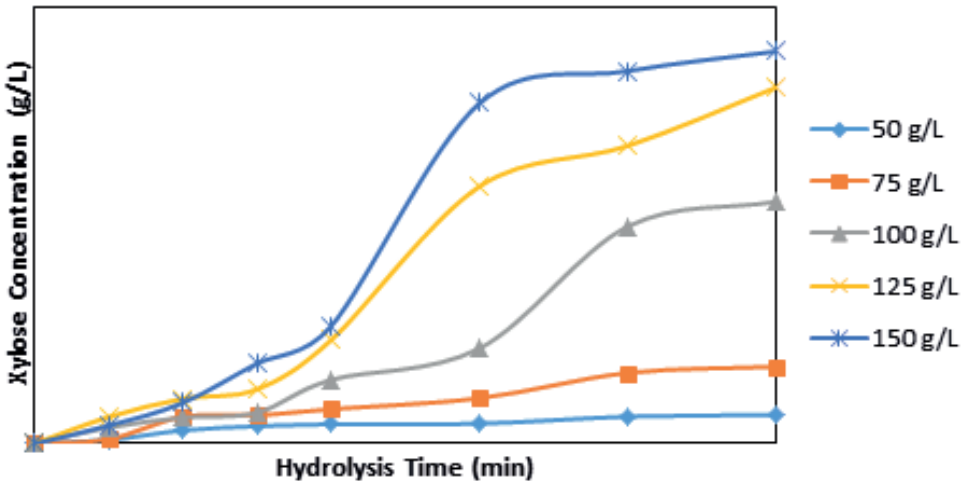

Figure 3: Influences of Initial OPP Concentration g/L) on the dynamic profile of xylose concentration.

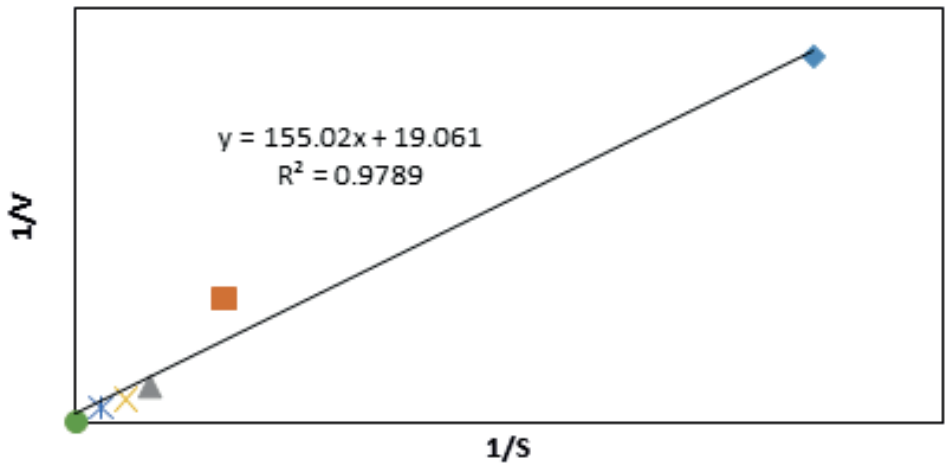

Figure 4: Lineweaver-Burke plot correlation between substrate (1/S) and reaction rate (1/V) in the enzymatic hydrolysis OPP.

4. Conclusion

The optimization of enzymatic hydrolysis of oil palm petioles was observed using response using xylose concentration as response, it was obtained at condition 50 °C, pH 4.8. pH and temperature significantly influenced xylose concentration on the hydrolysis process. Substrate concentration of oil palm petioles significantly affected the process and the enzymatic hydrolysis could be well expressed by the Michaelis Menten kinetic model, with K_m = 6.433 g/L and V_m= 0.042 g/L/min.

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