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Enyzmed Pretreated Empty Palm Fruit Bunch for Biofuel Production

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Abstract: Lignin peroxidase (LiP) and manganese peroxidase (MnP) enzymes were used to pre-treat empty fruit bunch (EPFB) before pyrolysis. Statistical analysis indicated that at 71.6%, LiP demonstrated greater lignin degradation compared to 67.9% MnP. Interestingly, the pretreatment sample has resulted in higher bio-oil yield compared to the untreated sample. Both LiP-treated and MnP-treated EPFB yielded approximately 30 wt% of bio-oil compared to 20 wt% of yield for the untreated sample.

Key words: Empty palm fruit bunch, lignin degradation, enzymatic pretreatment, pyrolysis, upgrading

INTRODUCTION

The palm oil industry is currently expanding rapidly and produces abundance of waste biomass including EPFB (Abdullah and Gerhauser, 2008). Recently, many research programs are focusing on the development of concepts such as renewable resources of energy for the alternative fuel. Lignin, the major component of biomass, is the most complex structures compared to hemicellulose and cellulose. It requires high temperature in pyrolysis process for biomass conversion. Therefore, enzymatic pretreatment had been introduced first before the pyrolysis process for degrading the lignin structures and obtaining higher yields with the improved properties of liquid products (Demirbas, 2007a).

Two groups of peroxidases, LiP and manganese peroxidase MnP enzymes have been reported as having a great potential in biological treatment for degrading lignin structures and it can turn into alternative fuels. This process has been extensively used in pulp and paper manufacturing as a pretreatment to pulping (biological), bleaching (bio bleaching), or wastewater treatment (Perez, *et al*., 2002). Regarding to Perez *et al.* (2002), LiP enzyme is more powerful oxidants than typical peroxidase are and consequently oxidize not only the usual peroxidase substrates but also a variety of non-phenolic lignin structures. Unlike LiP enzyme, MnP enzyme is not strongly oxidize and is consequently unable to attack non-phenolic structures that predominate in the lignin.

Pyrolysis process was found to be best suited for conversion of biomass to liquid fuel. Pyrolysis process is a thermo-chemical conversion process in the absence of air or oxygen (Demirbas, 2007b). Different conditions of the pyrolysis process have lead to the formation of products in different proportions of useful liquid oil, gases and solid (Goyal *et al.*, 2008). In this study, the pyrolysis experiment was performed by using a semi-batch reactor under nitrogen gas condition and HZSM-5 as the catalyst with the ratio of catalyst and EPFB, 5 wt%. The application of bio-oil as a fuel in boiler systems, stationary diesel engines, gas turbines and sterling engines had been widely reported (Junming *et al*., 2008; Mahfud *et al*., 2007). However, poor volatility, high viscosity, coking and corrosiveness of crude bio-oil have limited the applicability for the application mentioned above. Therefore, a number of bio-oil upgrading technologies were proposed to improve the product properties and to increase the range of possible applications such are hydrocracking or hydrodeoxygenation, which is highly expensive due to the use of hydrogen in the upgrading process (Mahfud *et al*., 2007).

In the present study, reactive distillation process was used to upgrade the pyrolysis oil. The purpose of the process was to improve the properties of oil including heating value, water content, viscosity and acid content. Water in the crude oil as well as the water produced by the various reactions was removed simultaneously as a distillate (Mahfud *et al*., 2007). The objective of this study is to investigate the potential of enzymatic pretreatment of EPFB for biofuel production. Pretreatment parameters which are enzyme ratio, reaction time and pH were optimized. The lignin degradation was determined by analyzing the Kappa Number via UV-Spectrophotometer.

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The EPFB samples were then catalytically pyrolyzed while the resulting bio-oil was further upgraded through reactive distillation.

MATERIALS AND METHODS

Materials: The EPFB was obtained from Felda Bukit Besar, Kulai, Johor. The EPFB samples were shredded and crushed to give particle size ranging from 0.1-0.5 cm. The proximate properties of the raw material are shown in Table 1 (Ani, 2001). LiP and MnP enzymes were supplied by Sigma Aldrich Company (Germany).

Experimental design: The optimal design for lignin degradation was conducted by using central composite design with full 2^3 factorial designs (three factors each at two levels). The factors were EPFB:enzyme ratio, reaction time and medium pH (Table 2). The levels of independent variables for each factor were used for the optimization of biological treatment (Table 3). The experimental data were analyzed using a STATISTICA version 6 software (StatSoft Inc., Tulsa USA).

Table 1: Main characteristics of EPFB (Ani, 2001)

Proximate analysis (wt%)	
Cellulose	59.7
Hemicellulose	22.1
Lignin	18.1
Ash	5.36
Moistures	7.48-8.96
Elemental analysis	
Carbon	47.89
Hydrogen	6.05
Nitrogen	0.65
Oxygen	45.41
High heating value (MJ kg^{-1})	16.7405

Table 2: Experimental ranges and levels of independent variables

Factors	-α (-2)	-1	0	1	+α (+2)
Ratio (EPFB:enzyme), X_1	1:1,000	1:5,000	1:10,000	1:15,000	1:20,000
Reaction time, X_2	2	3	4	5	6
pH, X_3	4	5	6	7	8

Table 3: The experimental designs and lignin degradation performance in each run

	Independent variables			Dependent variable	
				Lignin Degradation (%)	
Standard run	Ratio (EPFB:enzyme)	Reaction time (h)	pH	Treated (LiP)	Treated (MnP)
1	5000	3.00	5.00	48.29	66.23
2	5000	3.00	7.00	61.63	49.80
3	5000	5.00	5.00	43.82	27.27
4	5000	5.00	7.00	26.02	17.61
5	15000	3.00	5.00	71.22	52.50
6	15000	3.00	7.00	67.21	54.60
7	15000	5.00	5.00	19.58	60.45
8	15000	5.00	7.00	28.65	57.16
9	10000	4.00	6.00	69.97	55.19
10	1180.83	4.00	6.00	49.61	14.19
11	18819.17	4.00	6.00	60.12	60.91
12	10000	2.24	6.00	51.12	54.73
13	10000	5.76	6.00	25.03	67.94
14	10000	4.00	4.24	45.07	29.04
15	10000	4.00	7.76	71.62	24.51

Experimental procedures

Pretreatment: Grinded EPFB was solubilized in 0.01 M NaOH before adjusting the pH to the intended value with 100 mM sodium tartrate for preparing tartrate buffer solution (Moreira *et al*., 2007). The appropriate amount of LiP enzyme was then added according to the desired sample ratio. The reaction was conducted at room temperature under stirring at 90 rpm for the appropriate pretreatment time. Meanwhile, the pretreatment of EPFB with MnP enzyme was carried out at the same conditions in malonate buffer solution at pH 5.0 with addition of 0.4 mL $MnSO_4$ (0.1 mM). At the end of the reaction, the pretreated samples were dried overnight at temperature being 100°C. UV-VIS analysis was employed on the sample for lignin content determination.

Pyrolysis: A well-mixed treated EPFB (50 g) with 5 wt% of HZSM-5 were placed together in semi-batch reactor. The catalytic pyrolysis was carried out in an inert condition at a fixed temperature of 300°C. The vapor produced from the reaction was forced to flow through a condenser and consequently condensed into liquid bio-oil.

Analysis methods: Direct spectroscopic pulp kappa test method was employed to determine lignin content in the sample (Chai and Zhu, 1999). The one-point calibration method would determine the value of Kappa Number, K as shown in Eq. 1. Accordingly, lignin content in the sample was calculated from the values of Kappa Number, K using Eq. 2, while the percent of lignin degradation is calculated in Eq. 3 (Ohra-aho *et al*., 2005).

$$K = \frac{\alpha}{w}\left(\frac{A_o - A_s}{A_o}\right) \quad (1)$$

$$\text{Lignin content (wt\%)} = 0.15K \quad (2)$$

$$\text{Lignin degradation (wt\%)} = \frac{\text{Lignin wt.\% (untreated)} - \text{Lignin wt.\% (treated)}}{\text{Lignin wt.\% (untreated)}} \quad (3)$$

where, α is the volume of $KNnO_4$ used in the solution, w is weight of moisture free sample used, A_o is spectral intensities at time t = 0 (before sample is treated) and A_o is spectral intensities at the end of the reaction.

The liquid products were analyzed using Gas Chromatography–Mass Selectivity Detector (Agilent, US) with the 30.0 m ×250 μm ×0.25 μm nominal column (Boateng *et al*., 2006; Wang *et al*., 2007). The initial, intermediate and final temperature programs were 80, 250 and 300°C, respectively while helium was used as a carrier gas at a flow rate of 0.2 μL.

RESULTS AND DISCUSSIONS

Pretreatment process: Lignin is the most difficult component of biomass to be degraded due to its complex structure, high molecular weight and high insolubility. Lignin is linked by carbon-carbon and ether bonds to form tri-dimensional network associated with the hemicelluloses polysaccharides inside the cell wall (Ibrahim *et al*., 2005). The delignification reactions involved the cleavage of non-phenolic β-O-4-linkage, phenolic α-O-4 linkages and releasing from the associated by the polysaccharide.

The capability of LiP and MnP enzymes in degrading lignin were investigated. Based on Table 3, the highest percentage of lignin degradation by LiP enzyme was observed in standard run 15 with 71.62% degradation where the ratio of enzyme to EPFB was 1 (kg):10,000 (μL), reaction time of 4 h and pH value 7.76. The pretreatment with MnP enzyme however, yielded 67.94% as the highest degradation at ratio 1(kg):10,000 (μL), reaction time of 5.76 and pH value 6. Obviously, higher lignin breakdown by LiP enzyme was observed.

It agreed well with the literatures that reported LiP enzyme as powerful oxidants than typical peroxidases enzyme due to its ability to oxidize both phenolic, non-phenolic structures and other aromatic ethers that resemble the basic structure unit of lignin (Jeffries, 1994; Hammel, 1997; Perez *et al*., 2002; Cullen and Kersten, 2004). In contrast, MnP was not strongly oxidized enzyme and unable to attack the non-phenolic structures that predominate in the lignin. The enzyme requires the presence of the chelator to stabilize the Mn (III). Mn (III) chelates to oxidize more reactive phenolic structures that make up approximately 10% of lignin (Hammel, 1997). The principal function of MnP is to oxidize Mn (II) to Mn (III) and in order to stabilize the Mn (III), manganese sulfate ($MnSO_4$) was used as the chelator in the pretreatment.

Optimization of lignin degradation: A polynomial regression equation was developed by using Analysis of Variance (ANOVA) to analyze factor interactions by identifying the significant factors contributing to the regression model. In addition, it was vital for the optimal value determination. The model equation for LiP and MnP enzymes pretreatment are shown in (4) and (5), respectively.

$$Y_1 = -361.146 + 0.009X_1 + 102.664X_2 + 65.278X_3 - 0.000X_1^2 - 11.123X_2^2 - 4.608X_3^2 - 0.001X_1X_2 + 0.000X_1X_3 - 2.258X_2X_3 \quad (4)$$

$$Y_2 = -82.8243 - 0.0061X_1 - 47.5229X_2 + 88.7367X_3 - 0.000X_1^2 + 2.9309X_2^2 - 2.9309X_2^2 - 8.1776X_3^2 + 0.0020X_1X_2 + 0.0006X_1X_3 + 0.1725X_2X_3 \quad (5)$$

where, Y_1 and Y_2 is the predicted lignin degradation by LiP and MnP enzymes, X_1 is the ratio of EPFB and enzyme (1 kg EPFB: enzyme (iL), X_2 reaction time and X_3 is the initial reaction pH.

From the regression equation above, the optimum value for the lignin degradation can be concluded at the critical values of the independent variables. The coefficient of determination (R^2) for respective LiP and MnP enzymes are 83 and 85%. Both were insured a satisfactory data and for a practical rule of thumb, the determinant coefficient, R^2 should be equal or greater than 75% (Haaland, 1989).

The main effect of independent variables on dependent variable was further investigated using Pareto charts (Fig. 1, 2). The length of each bar in the Pareto

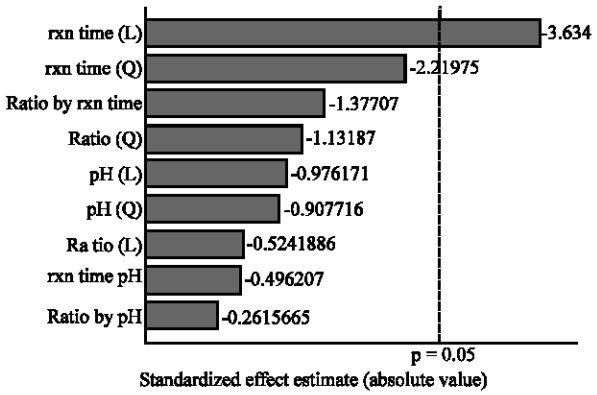

Fig. 1: Standard pareto chart showing the effects of independent variables and their combined effects on the lignin degradation, Y_1

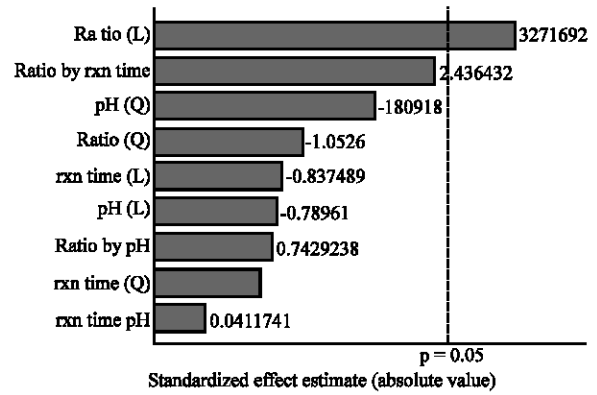

Fig. 2: Standard pareto chart showing the effects of independent variables and their combined effects on the lignin degradation, Y_2

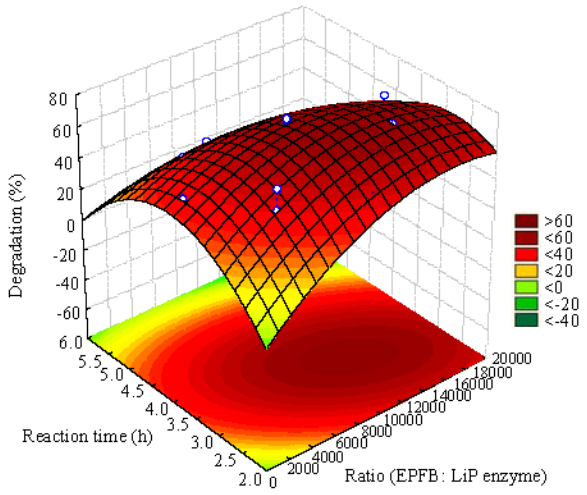

Fig. 3: The response surface plot of percentage lignin degradation by LiP enzyme as a function of ratio and reaction time at constant pH = 6

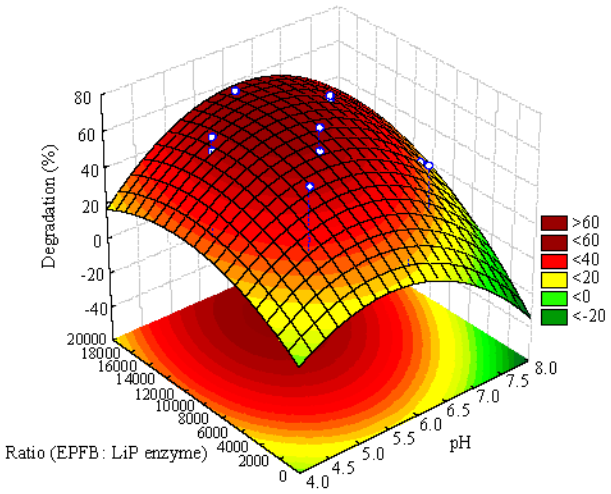

Fig. 4: The response surface plot of percentage lignin degradation by MnP enzyme as a function of ratio and pH at constant reaction time = 4 h

chart indicated the effect of factors (EPFB and enzyme ratio, reaction time, pH) on response (lignin degradation).

It was found that the reaction time factor has the major effect on the lignin degradation for LiP enzyme pretreatment, while the EPFB-enzyme ratio factor appeared the highest effect for MnP enzyme in lignin degradation. In the experimental analysis, all the independent variables affected the reaction response. However, different types of enzymes applied in the pretreatment process contributed to different effects of independent variables towards lignin degradation.

The relationship between the dependent and independent variables were further clarified using response surface plots. Figure 3 shows the response surface plot of percentage lignin degradation for LiP enzyme pretreatment as a function of ratio and reaction time when the initial pH is constant at pH 6. It was observed that the lignin degradation started to decrease beyond 4.5 h reaction time. From the process point of view, sufficient time is favorable to ensure complete reaction between the enzyme and lignin in the sample. Longer reaction time may possibly inhibit the activity of LiP enzyme. Meanwhile, higher EPFB-enzyme ratio would give higher lignin degradation. It was in accordance with literature where higher enzyme concentration would contribute to more reactions occurred and efficiently degraded lignin structure in the substrate. The enzyme may catalyse polymerization, chromophore alteration or breakdown of lignin molecules (Moreira *et al*., 2007).

Pretreatment with MnP on the other hand has demonstrated significant effect between ratio and pH towards lignin degradation. It was elucidated using response surface plots as shown in Figure 4. The relative amounts of EPFB and enzyme have the major effect on the reaction response. Both factors showed similar trend of lignin degradation where beyond the maximum point, decreasing in lignin degradation was observed. An increase in the amount of enzyme added per unit substrate led to a decrease in the lignin degradation. From the 3D plot in this figure also, it is clear that the appropriate pH for this pretreated is at neutral condition. This observation also compromise with the result obtained from pretreated EPFB by LiP enzyme.

Pyrolysis process: After the pretreatment step, both treated and untreated samples were pyrolyzed in order to evaluate the effects of pretreatment method on bio-oil production. The reaction has decomposed EPFB to generate vapors that further condensed to form liquid oil, non-condensable gaseous and char as solid by-product remained in the reactor. High conversion is favored to produce high oil yield. The condensate oil formed two layers of liquid phase that consisted of heavy and lighter fractions (Table 4). The bottom layer (heavier components) was light yellow while the upper layer (lighter components) was dark brown.

Figure 5 shows the effect of the untreated and treated samples on the char, liquid and gas product yields in pyrolysis reaction. Both treated samples managed to give higher yield of liquid compared to untreated EPFB. The liquid yields were 34 and 31 wt% for LiP-treated and MnP-treated, respectively. The pretreatment could have altered

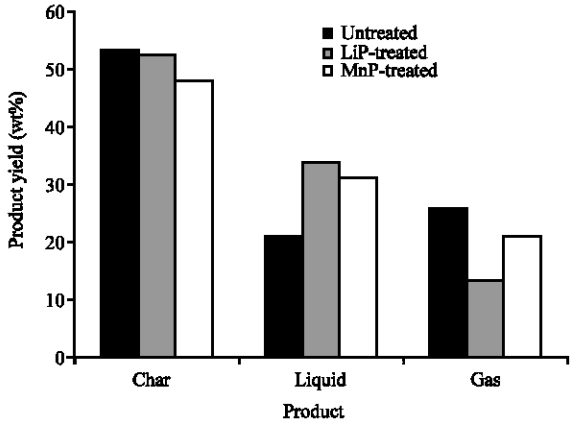

Fig. 5: The effect of untreated and treated samples on pyrolysis product yields

Table 4: Properties of bio-oil for untreated and treated EPFB

Properties	Untreated EPFB		Treated EPFB by LiP		Treated EPFB by MnP	
	Density (kg m^{-3})	pH	Density (kg m^{-3})	pH	Density (kg m^{3})	pH
Crude oil	990	2.64	1004	2.46	982	2.34
Upper layer oil	872	1.01	846	0.97	806	0.81
Bottom layer oil	1064	0.38	1038	0.34	986	0.35

the lignocelluloses structure and increased the accessible surface area of EPFB to be converted during pretreatment. Nevertheless, the LiP-treated EPFB yielded lower gas production namely 14wt%. The untreated sample however has produced high gaseous yield to compensate its low liquid yield product. Based on the results, all three samples demonstrated comparable yield of chars. These solid by-products could be further purified for the application of carbon black or activated carbon (Alam *et al*., 2007).

The percentage of conversion on the other hand was proven to be in the range of 45 to 50 wt%. Similar operating conditions may contribute to the close conversion difference between the samples. Reference Abdullah and Gerhauser (2008) reported higher EPFB conversion using fluidized bed reactor yielding 70 wt% of liquid and thus leaving char and gas yields around 15% each. Under fast pyrolysis, high liquid products were obtained at temperature of 500°C with short vapors residence times. In order to produce high yield, a very short residence time required. This happens because of incomplete depolymerisation of the lignin due to random bond cleavage and inters reaction of the lignin macromolecule. Longer residence times can cause secondary cracking of the primary products, reducing of liquid yield and adversely will affect the oils' properties (Bridgwater *et al*., 1999).

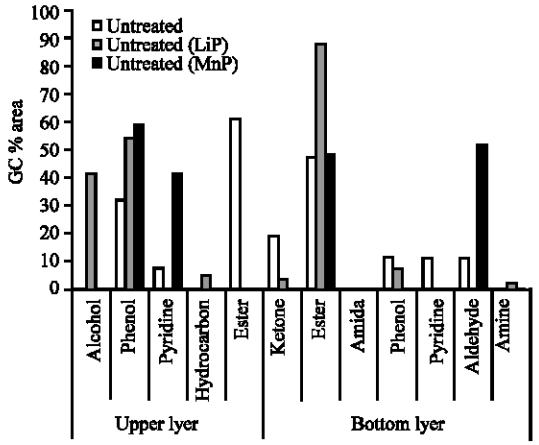

Fig. 6: Classification of compounds into different functional groups for upgrading bio-oils of untreated sample, LiP-treated sample and MnP-treated sample

The oil products were analyzed by GC-MS to determine the elemental composition of the oil. Figure 6 shows the classification of compounds into different functional groups for upgrading bio-oil of untreated sample, LiP-treated sample and MnP-treated sample. There is difficulty in determining the complete chemical characterization of oils. It is mainly due to the chemistry of bio-oil that can exhibit differences by changing the thermal conditions of the pyrolysis process.

All three samples were found to have mixtures of acids, alcohols, esters, ketones, aldehydes, phenols and its derivatives in its bio-oil product. The same trend was also reported in previous work (Demirbas, 2007a; Tamunaidu and Bhatia, 2007). Phenolic compounds are the main products obtained by this pyrolysis process. The presence of derivatives phenolic compounds as monomeric units and oligomers are derived by lignin structures in the EPFB.

CONCLUSIONS

In the pretreatment of EPFB by applied enzymes, LiP enzyme was seen as the more powerful oxidant compare to MnP enzyme. The percentage lignin degraded by LiP enzyme (71.69%) was higher than MnP enzyme (67.94%). EPFB to enzyme ratio was optimum in the range of 1 kg: 12500 μL to 1 kg: 13500 μL, while pH was 5 to 6 and reaction time was within 3 h. Treated EPFB produced higher conversion and liquid oil yield.

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