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Oil Palm Empty Fruit Bunch (OPEFB) Handsheet Production from Optimized Biodelignification of *Rhynchophorus Ferrugineus* Microbiome's Enzymes

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ABSTRACT

Oil palm plantation generates massive amount of oil palm empty fruit bunch (OPEFB) which source great amount of cellulose. However, wrapping this cellulose is an adhesive compound called lignin. Biodelignification process was applied to remove lignin in pulp and paper industry. Therefore, this study is focused on optimum conditions of delignification process using a combination of bacteria from *Rhynchophorus ferrugineus* on OPEFB. The composition of chemicals was characterized according to the TAPPI standard method and Kursher-Hoffner method. The Box-Behnken design (BBD) was used to determine the optimum conditions of delignification process based on lignin loss of OPEFB. The optimized fiber was investigated based on mechanical properties according to TAPPI standard methods. From BBD analysis, the finest conditions for delignification were recognized to be at 35 °C in 48 h incubation time with 5 mL of 1% glucose for predicted value 54.3% compared to experimental value 52% of lignin loss as revealed by confirmatory study. The highest result of chemical analysis was recognized at run 12 (1.15%), 10 (12.35%), 4 (48.99%) and 5 (1.28%) for extractive, lignin, cellulose and ash content respectively. The tensile, burst and tear were identified as 9.93 Nm/g, 0.98 kPa.m^2/g and 2.57 mN.m^2/g respectively for handsheet product at optimum conditions. In conclusion, the results obtained was indicated that the delignification process via bacteria combination from *R. ferrugineus* is a viable alternative pulping process for pulp and paper-based industry. The delignification process on OPEFB also provides a cleaner technology process and more sustainable development for the country.

Key words: Lignocellulosic; biodelignification; pulp and paper; lignin; *Rhynchophorus ferrugineus*.

1. INTRODUCTION

Palm oil (*Elaeis guineensis*) is a dominant agricultural crop in Malaysia. Over the last year, Malaysia has cultivated more than 5 million hectares of oil palm production and become one of the world largest exporters for palm oil. The Malaysia industry of oil palm had become the leading producers and exporters of palm oil, collectively generate large waste amounts of oil palm biomass. The oil palm empty fruit bunch (OPEFB) has become one of the massive waste productions with an annual value of 16 million tons [1].

The OPEFB was typically burned in palm oil mill incinerator and recycled ash content as the plantation fertilizers. However, OPEFB incineration has been discouraged due to the environmental issue [2]. The waste of lignocellulosic biomass must be used to ensure environmentally sustainable oil palm industry.

Lignocellulose biomass is the most abundant renewable biomass worldwide composed of hemicellulose, cellulose and lignin, including other minor components. Both hemicellulose and cellulose components are sugar polymers, hence potray possible source for fermentable sugars or other sugar product development. Inside the lignocellulose, lignin

fraction acts as a blockage towards microbial and enzyme penetration. Lignin significantly reduces fermentable sugar yields and adversely affects general energy production process from biomass resources to the extent that it is uneconomical [3]. To overcome this limitation, new types of biomass treatments are required for efficient production and economical [4].

Pulping was aimed at reducing cellulose crystanillinity, increasing the total surface area of biomass, breaking the lignin seal and removing hemicellulose, thereby increasing glucose yield. There are several major processes of treatment, for example mechanical and chemical treatments. Meanwhile, the biological treatment emphasizes strategies that harness microbes' natural metabolic activity to improve the biomass cycle potential on the agricultural residues. The leading process is the traditional pulping process such as chemical and mechanical pulping process, but they require significant energy inputs and often cause pollution from their effluents [5].

Biological pulping process uses less energy and chemicals, more economical and more environmentally sustainable [6], as compared to traditional pulping process. To date, the biological pulping process employs certain microorganisms, including white-rot and brown-rot fungi to deconstruct recalcitrantcy of lignin [7]. Although the successful fungi application in the biopulping processs has been observed, however, it is sensitive towards shearing and requires long incubation time (up to 60 days) which significantly reduces the lignin content [8]. Therefore, to overcome this problem, bacteria are introduced as an agent in the delignification process of lignocellulosic material.

Therefore, in this study, the optimisation of delignification conditions by using *Rhynchophorus ferrugineus* microbiome's enzymes on OPEFB was explored to significantly remove the lignin content in the waste materials. Henceforth, the objectives of this study are (i) to characterize the chemical properties of OPEFB prior to, (ii) optimise bioprocessing conditions of bacterial on OPEFB using Box-Behnken design (BBD) and (iii) to produce paper handsheet and determine its mechanical properties suitable for pulp and paper production.

2. MATERIALS AND METHOD

2.1 Bacteria Species

our major gut microbiome species having the ability to degrade lignin have been isolated from *R. ferrugineus* larvae and identified as *Klebsiella sp.*, *Enterobacter sp.*, *Serratia sp.*, and *Pseudomonas sp.* from previous research were used in this experiment [9]

2.2 Preparation of OPEFB

OPEFB was obtained from Pertubuhan Peladang Negeri Johor (PPNJ), Kahang, Kluang, Johor. Prior to use, the biomass was cleaned with tap water to remove soil and other impurities and sun dried until the water content was 10% or less. The dried sample was stored in plastic bags at room temperature.

Dried OPEFB was grounded to particle size about 0.4 mm and sieved through (40 mesh). Subsequently, the moisture content of powder OPEFB fiber was measured using moisture analyser [10]. If the moisture content is above 12%, the powder must be dried in a circulated oven at 40 °C for 24 hours.

2.3 Inoculum Preparation

Isolated bacteria species of *Klebsiella* sp., *Enterobacter* sp., *Serratia* sp., and *Pseudomonas* sp. were grown and incubated on LB agar overnight at 37 °C. After incubation, a single colony from every plate was transferred into LB broth media and incubated 4 to 6 hours at 37 °C. Then, each inoculated bacteria was added and mix with ratio 1:1:1:1 and incubated again in a temperature-controlled still culture incubator at 37 °C for 4 to 6 hours to obtain a homogenous colony forming of bacteria (1×10^8 CFU/mL) to be used as inoculum [9].

2.4 Optimization of Biodelignifiaction Process

2.4.1 Preparation of Biodelignification

Submerged fermentation is used for biological treatment of substrate in flask. The specimens (OPEFB) were heated for sterilization in autoclave at 120 □ for 15 minutes. After cool it was mixed with a suspension of bacteria (LB broth) and incubated based on the experimental design as presented in Table 1. After incubation period, the samples were heated again for sterilization at 120 □ for 15 minutes and were collected from the flask. Then, the samples were rinsed with distilled water, dried overnight at 40 □ prior to chemical analysis [11].

2.4.2 Experimental Design: Box-Behnken Design (BBD)

OPEFB's biodelignification process was optimized using Response Surface Methodology (RSM). The influence of three independent variables, A (incubation time, h), B (temperature, °C) and C (glucose volume, mL) at three levels (-1, 0, 1) on lignin loss were investigated using BBD and the coded and actual value of experimental design are shown in Table 1.

Table 1: Independent variables and their coded and actual level

Factor	Unit	Symbol	Coded level		
			-1	0	1
Incubation time	h	A	48	108	168
Temperature	°C	B	25	30	35
Glucose volume	mL	C	5	15	25

2.4.3 Chemical Characterization of OPEFB Fiber

All methods used for chemical analysis of OPEFB fiber were summarized in Table 2.

2.4.4 Handsheet Making and Mechanical Properties of Handsheet

Table 3 presents all standards for handsheet making and mechanical testing of handsheet from OPEFB.

Table 2: Method for chemical analysis of OPEFB fiber

Chemical Content Analysis	Standard Method	References
Extractives	Wood preparation for analysis of chemical	[12]
Holocellulose	Chlorite method	[13]
Cellulose	Kurscher-Hoffner method	[14]
Hemicellulose	Chlorite method	[13]
Lignin	Lignin acid-insoluble in wood and pulp	[15]
Ash content	Ash in wood, pulp, paper and paperboard: combustion at 525^{o}C	[16]

Table 3: Methods for handsheet making and mechanical testing

Handsheet making and mechanical testing	Standards Method	References
Handsheet Making	Handsheets formation for pulp physical tests	[17]
Preparation of specimen	Pulp handsheets physical testing	[18]
Tensile strength	Tensile properties of paper and paperboard (using constant rate of elongation apparatus)	[19]
Tearing resistance	Internal tearing resistance of paper (Elmendorf-type method)	[20]
Bursting resistance	Bursting strength of paper	[21]

3. RESULTS AND DISCUSSIONS

3.1 Chemical Characterization of OPEFB Fiber

Table 4 presents the summary of chemical compositions for OPEFB fibre before and after treatment with different conditions (incubation time, temperature and 1% glucose) under BBD.

The chemical compositions of OPEFB before treatment are extractive content (3.60 ± 0.14%), lignin content (18.90 ± 0.85%), holocellulose content (77.53 ± 0.18%), cellulose content (42.46 ± 0.19%), hemicellulose content (34.58 ± 0.33%) and ash content (2.02 ± 0.08). Whereas the best chemical compositions of treated OPEFB were obtained in different run such as extractive content (1.15 ± 0.14%, Run 12), lignin content (12.35 ± 0.35%, Run 10), hocellulose content (86.55 ± 0.21%, Run 9), cellulose content (48.99 ± 0.13%, Run 9), hemicellulose content (37.38 ± 0.32%, Run 9) and ash content (1.28 ± 0.11%, Run 5).

Generally, cellulose is significant suitable parameter for the material measurement for pulp and paper production [22]. However, the high lignin content in the material can decrease the performance of the pulp and paper product that caused the paper products to yellow [23]. Therefore, Run 10 is the best conditions for chemical compositions of OPEFB as shown in Table 4.

Table 5 illustrates the chemical composition of OPEFB with other non-wood, hardwood, softwood and annual plants, which have been successfully used in pulp and paper-based industry. The cellulose content of OPEFB (47.98%) was high and comparable with canola straw (42%), rice straw (41.20%), *C. orientalis* (40.10%) and *C. tataria* (40.30%) as shown in Table 5. In addition, the cellulose content above 40% was obtained in range of hardwood, softwood and annual plants, used to produce high quality of pulp and paper [24], [25]. According to Bidin *et al.* [25], the amount of cellulose influences paper strength and makes the fiber strands vulnerable to natural and synthetic dye binding.

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3.2 Optimization of the Biodelignification Process

A study of biodelignification process from *R. ferrugineus* microbiome's enzymes was evaluated by using RSM. The BBD was used in this study for the optimisation of delignification process by *R. ferrugineus* microbiome's enzymes on OPEFB. The effect of three independent variables such as incubation time (A), temperature (B) and 1% glucose volume (C) were investigated as shown in Table 6. Overall, 15 experimental runs with 3 replicates were performed on the experimental results value and predicted value by the model presented in Table 6.

The highest lignin loss was observed in Run 11 with average lignin loss of 52% with the conditions of 108 h incubation time, 35 °C temperature and 5 mL 1% glucose volume. Meanwhile, the lowest percentage of lignin loss was found in Run 4 with average of 29.7% at 48 h of: incubation time, 30°C temperature in 5 mL of 1% glucose volume.

Table 4: Chemical compositions of OPEFB fiber

Sample conditions	Extractive (%)	Lignin content (%)	Holocellulose content (%)	Cellulose content (%)	Hemicellulose content (%)	Ash content (%)
Before Treatment	3.60±0.14	18.90±0.85	77.53±0.18	42.46±0.19	34.58±0.33	2.02±0.08
1	1.58±0.04	15.10±0.28	81.50±0.75	45.77±0.41	35.94±0.68	1.41±0.14
2	2.58±0.11	13.95±0.07	81.80±0.14	45.95±0.64	35.50±0.00	1.71±0.34
3	2.18±004	13.45±0.07	78.55±0.35	47.90±0.28	30.40±0.28	1.31±0.30
4	2.45±0.04	18.40±0.42	79.30±0.07	48.99±0.13*	30.71±0.76	1.53±0.23
5	1.78±0.04	14.50±0.07	78.08±0.81	47.33±0.64	30.67±0.11	1.28±0.11*
6	1.58±0.04	15.65±0.07	81.50±0.42	46.65±0.35	35.30±0.71	1.55±0.02
7	2.73±0.11	15.95±0.21	79.45±0.07	46.81±0.78	33.28±0.05	1.74±0.08
8	2.88±0.04	13.75±0.21	78.75±0.42	47.27±0.80	31.29±0.09	2.27±0.97
9	1.43±0.04	16.95±0.07	86.55±0.21*	48.53±0.81	37.38±0.32*	1.61±0.11
10	1.85±0.14	12.35±0.35*	78.88±0.04	47.98±0.46	31.62±0.52	1.65±0.12
11	3.33±0.32	13.85±0.07	75.10±0.85	48.33±1.56	26.15±3.29	1.67±0.05
12	1.15±0.14*	15.20±0.14	78.83±0.25	48.90±0.67	30.40±0.42	1.74±0.04
13	2.38±0.04	15.15±0.07	82.60±0.35	48.43±0.78	34.94±0.66	1.87±0.04

*best values for pulp and paper based parameters

Table 5: Chemical compositions of OPEFB and other published non-wood, hardwood, softwood and annual plants

Materials	Chemical compositions, %					References
	Cellulose	Hemicellulose	Lignin	Extractive	Ash	
OPEFB	47.98*	31.62*	12.35*	1.85*	1.65	This study
Canola stalk	42	31.6	17.3	8.2	2.5	[26]
Rice straw	41.2	19.5	18.1	9.2	0.56*	[27]
C. orientalis	40.1	30.4	24.5	6.26	7.83	[28]
C. tataria	40.3	29.7	20.59	5.04	9.31	
Hardwoods	38 - 55	n/a	18 - 26	1-Jun	0.2 - 0.7	[29]
Softwoods	55 - 61	n/a	25 - 32	1-Jun	0.2 - 0.7	
Annual plants	40 - 52	35 - 47	15 - 19	5-Sep	2-Jul	

n/a = not available; *best values for pulp and paper-based parameters

To validate the statistical results and the model equation, analysis of variance (ANOVA) was performed as shown in Table 7. Moreover, the result of the degree of freedom (DF) is 9 which is equivalent to the independent observation number. The *p* value can be taken as factor for significant verification of each coefficient which explains the interaction between the strength of each parameter and mutual interaction pattern between variables [30]. Several criteria were used to check goodness of fit of the model. The *p*-value less than 0.05 shows that the model term are significant as shown in Table 7. In this case, B, C, A^2, C^2, AB, AC and BC had a significant effect ($p < 0.05$) on lignin loss of OPEFB. Lack of fit *F* value 48.22 implies that the lack of fit is significant. The terms A and B^2 seems to be insignificant, which can be neglected from the model without affecting goodness of the fit [31].

Table 8 illustrates the ANOVA for regression quadratic model analysis by BBD. The determination coefficient (R^2) value 0.9372 indicates that the model was able to explain 93.72% of the variability in the response. In addition, the value of R^2 also is presented a correlation between the predicted and the experimental values [30], [31]. Also the adjusted determination coefficient (adj. R^2 = 0.9210) was high, indicating the significancy of the model [31].

Table 8: ANOVA for the regression model

Source	Value
Standard deviation	1.8
R^2	0.9372
Adjusted R^2	0.921
Predicted R^2	0.8895

The determination of high lignin loss with optimal levels of incubation time (A), temperature (B) and 1% glucose (C) was inferred with second-order polynomial model for evaluating the relationship between dependent and independent variables. The following second-order polynomial equation based on the multiple regression analysis explained between variables and lignin loss OPEFB:

$$\text{Lignin loss } \% = 38.0333 + 1.2454B + 0.9714C - 4.0500^2 + 2.85383^2 - 3.600AB + 6.0971AC - 7.917BC \quad (1)$$

where A, B and C are the independent variables.

To gain a better understanding of the effects of the variables on the loss of lignin, the predicted model has been presented as a plot of the response surface**.**

3.3 Incubation Time vs. Temperature

Figure 1 illustrates the interaction between incubation time, temperature and their reciprocal interactions with the lignin loss percentage from the delignification process. This response surface plot was generated holding at 1% glucose volume. As shown in Figure 1, both the incubation time and temperature had positive impact on the delignification process. An increase of the lignin loss can be observed with the reduction of incubation time and the increases of temperature applied to OPEFB delignification.

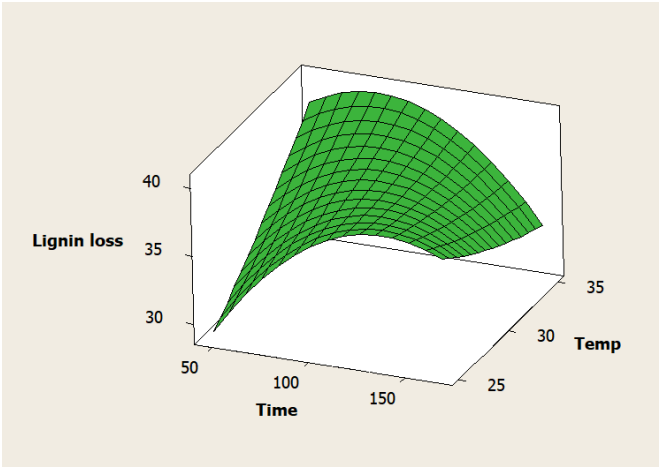

Figure 1: Response surface plot for the interaction between incubation time and temperature on lignin loss (hold values: 1% glucose volume, 15 mL)

3.4 Incubation Time vs. 1% Glucose Volume

Figure 2 shows the interaction between incubation time and 1% glucose volume with temperature at 30 °C. As shown in Figure 2, both the incubation time and 1% glucose volume have synergistic effect on the delignification process. An increase of the lignin loss can be observed with the increases of incubation time and 1% glucose volume applied to OPEFB fibre delignification. In addition, the low lignin loss was obtained at high incubation time (168 h) and low 1% glucose volume (5 mL).

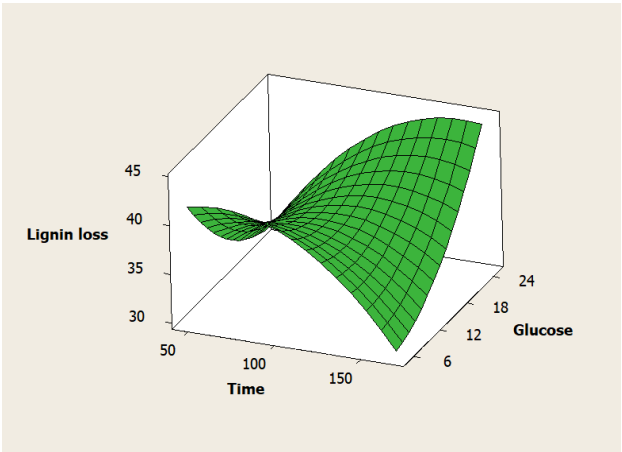

Figure 2: Response surface plot for the interaction between incubation time and 1% glucose volume on lignin loss (hold values: temperature, 30°C)

Table 6: BBD matrix for delignification process of microbiome's bacteria from *R. ferrugineus*

Run	Experimental factors			Response: Lignin loss, %		Error (%)
	Incubation time, h	Temperature, °C	1% glucose, mL	Actual value	Predicted value	
1	168	30	25	46.1	45.1	2.2
2	108	30	15	38	37.6	3.3
3	168	25	15	38	40.4	-6.4
4	168	30	5	29.7	31	-4.5
5	48	35	15	38.1	42.1	-10.6
6	108	25	5	31.1	27.6	11
7	108	35	25	36	32	11.1
8	168	35	15	30.4	35.7	-17.3
9	108	30	15	38	37.6	3.3
10	48	25	15	31.2	32.3	-3.5
***11**	**108**	**35**	**5**	**52.1**	**45.7**	**12.2**
12	108	30	15	38	37.6	3.3
13	48	30	5	39.8	42.4	-6.6
14	48	30	25	31.8	32.1	-0.9
15	108	25	25	46.2	45.1	2.4

Table 7: ANOVA for the response surface quadratic model

Source	Sum of squares	DF	Means square	*F* value	*p* value	Remarks
Model	1689.32	9	187.702	58.03	0	Significant
A	4.08	1	4.084	1.26	0.269	
B	37.75	1	37.75	11.67	0.002	Significant
C	21.28	1	21.282	6.58	0.015	Significant
A^2	205.77	1	181.689	56.17	0	Significant
B^2	0.59	1	2.243	0.69	0.411	
C^2	90.5	1	90.499	27.98	0	Significant
AB	155.52	1	155.52	48.08	0	Significant
AC	445.3	1	445.301	137.67	0	Significant
BC	728.52	1	728.521	225.23	0	Significant
Residual error	113.21	35	3.235			
Lack of fit	92.7	3	30.9	48.22	0	Significant
Pure error	20.51	32	0.641			
Total	1802.53	44				

A = Incubation time, B = Temperature, C = 1% glucose, DF = degree of freedom, *f* = Fisher's function, *p* = corresponding level of significance ($p<0.05$)

3.5 Temperature vs. 1% Glucose Volume

Figure 3 presents a response surface plot of temperature against 1% glucose volume at fixed level of time (108 h). As shown in Figure 3, the lignin loss increased at low temperature (25 °C) and increased at high 1% glucose volume (25 mL). In contrast, lignin loss decreased at low temperature and low volume of 1% glucose. This similar with interaction at high temperature and high volume of 1% glucose.

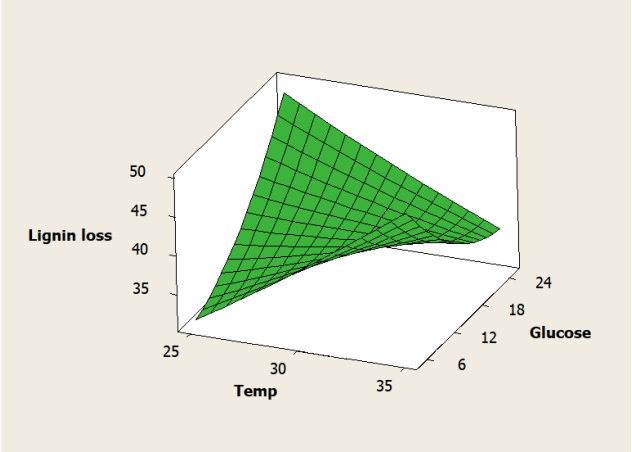

Figure 3: Response surface plot for the interaction between temperature and 1% glucose volume on lignin loss (hold values: time, 108 h)

3.6 Optimization Conditions

From the response surface plots, it can be observed that each of the three variables used has its own individual effects on lignin loss by *Rhynchophorus ferrugineus* microbiome's enzymes in bio-delignification process. From the BBD analysis, the optimal values were obtained 48 h for incubation time at 35°C of temperature with added 5 mL of 1% glucose volume. At these optimal conditions, the predicted lignin loss of biodelignification process was 54.68%.

3.7 Mechanical Properties of Handsheet

The mechanical properties (also known as strength properties) of handsheets can serve as indicator to determine the characteristic of handsheet product from non-wood or wood resource involved in the paper-making industry. Monitoring of mechanical properties of handsheet was carried out to evaluate the effectiveness of delignification process of OPEFB fibers. In this study, the mechanical properties of OPEFB handsheet were focused on the tensile strength, tearing resistance and bursting strength. Handsheet with higher values in mechanical properties could be selected as alternative pulping process in pulp and paper industry. Therefore, the test on mechanical properties is conducted on handsheets from untreated and treated OPEFB. The treated sample is the sample produced after delignification process at optimization condition.

Figure 4 shows the value of tensile index of handsheet for untreated and treated OPEFB. The highest value of tensile index was obtained in treated OPEFB (9.93 Nm/g) under biodelignification process compared to untreated OPEFB (9.18 Nm/g). The error bars are small indicating good accuracy and precision of repeatability results in this study. The high value of tensile index in treated OPEFB was proven that the biodelignification process is comparable for pulp and paper-based industry.

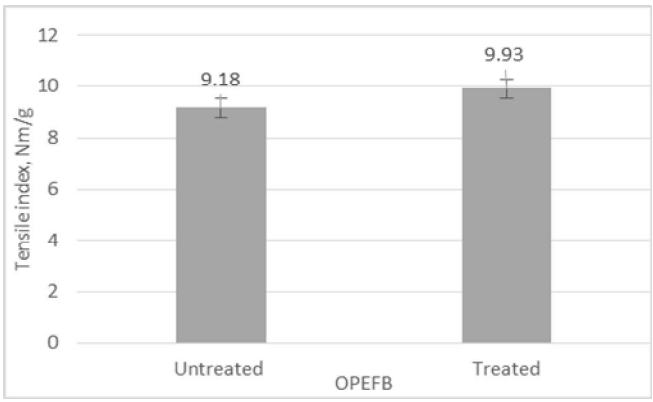

Figure 4: Tensile index of handsheet for untreated and treated OPEFB

In pulp and paper industry, tensile strength is one of the important mechanical parameter in describing the resistance of the paper product [32]. The tensile strength is also an indicator to fiber strength, fiber bonding and fiber length of material [33]. In addition, increase of tensile index also depends on the contact area of inter fiber bonding [33]. Table 9 presents the comparison tensile index of OPEFB with other published materials. OPEFB shows the low tensile index (9.93 Nm/g) compared to poplar wood (42.70 Nm/g), which OPEFB have low cellulose content than poplar wood that affects the strength and contact area of fiber interbonding [24], [34]. Interestingly, when comparing to other published material, it is significantly higher than vine straw and giant reed node (Table 9) which has been successfully applied in pulp and paper-based industry.

Table 9: Comparison of tensile index value of OPEFB and other non-wood and wood plants species

Materials	Tensile index, Nm/g	References
OPEFB	9.93	This study
Vine straw	6.45	[35]
Giant reed node	5.2	[36]
Hardwood	45 - 54	[37]

Figure 5 shows the value of tear index of handsheet for untreated and treated OPEFB at optimum condition. When comparing between untreated (2.52 $mN.m^2/g$) and treated

(2.57 mN.m^2/g) OPEFB, it appears that tear index are relatively close. Normally, tear index decreases with increasing proportion of fiber length in material [38]. Fiserova and Gigac [34] stated that less interbonding may be correlated with the decreasing number of bonds (hydrogen bonds) formed by the material of hemicellulose. From this study, the tear index of untreated and treated OPEFB are quite similar due to the hemicellulose content is not significant difference after biodelignification process.

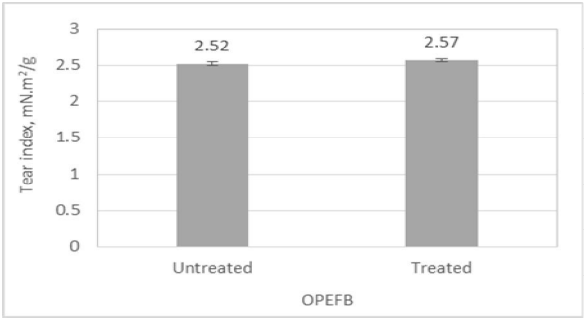

Figure 5: Tear index of handsheet for untreated and treated OPEFB

Table 10 presents the difference of tear index obtained in OPEFB and other published non-wood and hardwood plants. Interestingly, OPEFB has the highest tear index than non-wood and hardwood plants (Table 10). This is due to the high fiber bonding as indicated high hemicellulose content and low lignin content of material [33].

Table 10: Comparison of tear index value of OPEFB and other non-wood and wood plants species

Materials	Tear index, mN.m^2/g	References
OPEFB	2.57	This study
Vine straw	0.9	[35]
Rice straw	1.2	[27]
Cogon grass	2.17	[23]
Hardwood	1.09	[39]

Figure 6 illustrates the comparison burst index between untreated and treated OPEFB. The treated OPEFB shown the high burst index (0.98 kPa.m^2/g). This was shown successfully the biodelignification process by *Rhynchophorus ferrugineus* microbiome's enzymes on OPEFB under optimum condition.

Table 11 reported that the burst index obtained from OPEFB compared to other published nonwood and hardwood plants. Although OPEFB has low burst index than vine straw, rice straw and hardwood, however, it has the high burst index than published oil palm leaves (Table 11) that have been successfully used in pulp and paper industry.

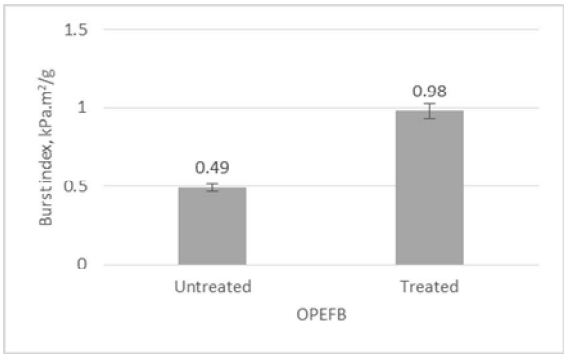

Figure 6: Burst index of handsheet for untreated and treated OPEFB

Table 11: Comparison of burst index value of OPEFB and other non-wood and wood plants species

Materials	Burst index, kPa.m^2/g	References
OPEFB	0.98	This study
Vine straw	1.01	[35]
Rice straw	1.2	[27]
Oil palm leaf	0.95	[40]
Hardwood	1.4	[41]

4. CONCLUSIONS

The purpose of this research is to determine the best condition of delignification process as an alternative process in pulp and paper-based industry, which focuses on the lignin loss of the OPEFB. The characterization of chemical properties of OPEFB fiber prior to treatment and after treatment was carried out successfully. The chemical properties of OPEFB at the best conditions by BBD such as cellulose content (47.98%), hemicellulose (31.62%), lignin content (12.35%), extractive content (1.85%) and ash content (1.65%) indicate OPEFB fibre is a biomass for pulp and paper-based product.

Next, the optimization of bioprocessing conditions of *R. ferrugineus* microbiome's enzymes on OPEFB was elicited in the experimental design. Based on the BBD, the experimental value shows the higher lignin loss is 52% at 35 °C temperature for 108 h incubation time in 5 mL of 1% glucose. The model was evaluated for selecting the optimal conditions and their respective levels. The final optimized biodelignification process conditions obtained from BBD is 54.68% of lignin loss at 35 °C temperature for 48 h incubation time in 5 mL of 1% glucose.

Based on the mechanical properties, the overall handsheet produced by treated OPEFB fiber yield better quality pulp compared to untreated OPEFB fiber, but still low compare to hardwood plants resources. The mechanical properties values of treated OPEFB fibre with optimum delignification process

are tensile index (9.93 mN/g), tearing index (2.57 mN.m2/g) and burst index (0.98 kPa.m2/g).

The results of this study provide an understanding on feasibility of biodelignification process by *R. ferrugineus* microbiome's enzymes on OPEFB. The statistical optimization method helped to define the most critical operational variables and optimum rates led to the highest loss of lignin. In addition, it also offers a better alternative pulping process than using fungal and conventional mechanical and chemical treatments in pulp and paper-based industry. This biodelignification is expected to provide a cleaner technology.

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