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Research paper

Bio-Mechanical Pulping of Bacteria Pre-Treatment on Oil Palm Biomass for Handsheet Production

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Abstract

Biopulping is one of the alternative process towards conventional process; chemical and mechanical processes. The biopulping process in this study was delignification using the bacteria Bacillus sp. which were isolated from the *Coptotermes curvignathus* gut (termite insects). The research was explored to determine the best performance of the bacteria species on the oil palm biomass in handsheet production. The bio pulp was produced by biopulping under submerged fermentation using luria broth (LB) at pH 6.5, 35°C for 7 days. The bio pulp samples were grinded using refiner mechanical pulping (RMP) which were sieved through 200 µm, furthermore proceeded with the handsheet production according to technical association pulp and paper industry (TAPPI) standard method. The characterization of handsheet physical properties were analysed on tensile, bursting, tearing, brightness and opacity. From this result, EFB treated shows better performance in mechanical strength with low grammage value of 48.952 g/m^2, 637.3 µm thickness average, 7.144 Nm⁄g tensile index, 1.6850 $Mn.m^2/g$ tearing index and 0.346 $kPa.m^2/g$ of bursting index. Therefore, this bacteria shown to be an alternative process for greener and environmental friendly production of pulp and paper industry and the non-wood empty fruit bunch of oil palm have greater potential in replacing wood resources.

***Keywords**: Bio-delignification, Bio-mechanical pulping, termite guts bacteria, papermaking and pulp properties*

1. Introduction

The pulp and paper industry (PPI) produces pulp, paper, board and other cellulose-based products which gone through the process of pulping, papermaking and paper finishing. Over the years, pulp and paper industry has known to be the fourth largest industrial energy user worldwide. In 2010, the global production of paper was 394 million tons [1,2]. Over half of this production took place in Europe (28%) and North America (23%), while in Asia it was about 42% of the total paper production. On the other hand, a relative low share in world paper production could be found in countries of South America (5.2%) and Africa (1.1%). Asia has the largest share in total paper production (42%), followed by Europe (28%) and North America (23%) [2]. The paper global demand is expected to grow about 3% annually in the period of 15 years since 2005. While in the Asia region, China is the top producer and consumer of paper-based product which is over 100 million metric tons followed by United States (72 million metric tons), and Japan (27 million metric tons) [1]. Despite the demand rise for paper products, there is limited raw materials which obstruct the development of the production of products in pulp and paper industry.

In Malaysia, the total capacity of paper and pulp production has been reported by Asia Pro Eco program (2006) that over 1 million metric tons per year has been produced and suspected to be higher by years to come. This demand in wood sources for the pulp and paper production from forest lands and plantation has decreased due to worldwide plantations and especially in Malaysia Oil Palm Plantation. In this case, the Oil Palm plantations expansion in the last few decades has become the biggest cause for deforestation in Malaysia [3]. The google deforestation map for the year 2000-2012 (e.g. figure 1) shows that Malaysia and Indonesia has the highest deforestation rate of forest loss over its land shown in figure 2, thus this has caused an import demand of wood supply from overseas in order to fulfil the local demand [4].

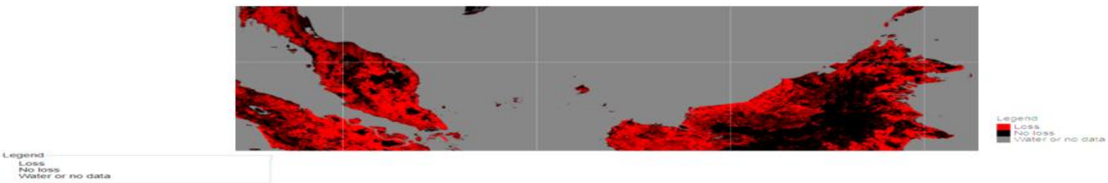

Fig. 1: Google Map View of forest loss in Malaysia, 2000-2012[4].

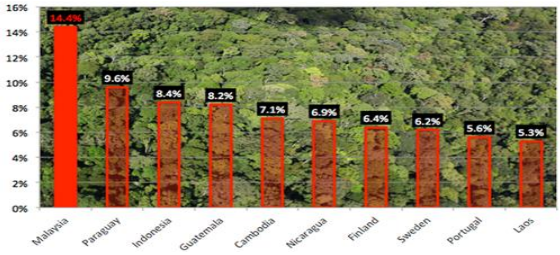

Fig. 2: Percentage of major forest loss in 2000-2012 [5]. Retrieved from https://news.mongabay.com/2013/11/malaysia-has-the-worlds-highest-deforestation-rate-reveals-google-forest-map/

In order to provide paper-based products, there has been several downsides that caused environmental problems, costly expenditure and high energy consumption due to the highly use of chemicals and electrical power [6,7,8]. Generally, pulp and paper productions are mostly produced based on fibrous raw material which may contain primary and secondary fibres. Primary fibres are obtained directly from raw plant material, mainly from wood or non-wood plants whereas secondary fibre is obtained from recovered paper such as recycled paper [1]. About 90% of the conventional raw material for the pulp and paper production worldwide are from wood [8] mostly in the developed countries [9,10] such as United State of America, USA and Canada [11]. Generally, the pulp and paper industries obtain cellulose from hardwood or softwood materials which are high in demand compaed with non-wood materials [12].

Furthermore, the high market demand has also cause the usage of large number of wood supply in pulp and paper production which rises the idea of interest to seek for non-wood plants as substitution fibre that is more economical and sustainable [13,14]. Therefore, non-wood resources including agricultural residues such as cogon grass, oil palm leaves [15] empty fruit bunch [16], rapeseed straw [17], kenaf [16,18,19] and wheat straw [14] could be the source for paper and pulp production. According to Daud and Law (2011), oil palm biomass was strongly suggested for the base of paper and pulp production since Malaysia has been one of the largest base of oil palm plantations that enormously produced 70 million tonnes of oil palm biomass of fronds, trunks and empty fruit brunches [20,21].

The papermakers from North American and Europe are now seeking for alternatives using non-wood fibres materials for the production reinforcing an opportunity for oil palm producing countries, especially Malaysia to utilize the biomass waste to be used in paper and pulp industry [20,21].This is due to the level of oil palm production has a bigger amount of waste either from plantations (10%) or palm oil industries (90%) which could contribute for the alternative resources [13]. During last 4 decades, the limit of the non-wood plant fiber pulping has expanded on a worldwide premise a few times as quick as the wood pulping capacity [22,23]. Non-woods have been utilized as the primary crude materials for paper preparations in numerous nations, including China and India. Therefore, research and growth of a few non-wood-based crude materials for pulp and paper have been attempted quickly by specialists in Malaysia. Malaysia's vast oil palm industry deposits have been used by numerous researchers as non-wood based paper building material [20]. Paper making deposits of palm oil industries include empty oil palm fronds, oil palm male flower spikes and empty fruit bunch. [23].

Pulp delignification is a crucial phase for paper making in industries. The refractory nature of lignin compound towards degradation makes it hard for the pulp based industries to efficiently remove lignin during pulp-processing. In order to produce high quality fibres utilizing all component of wood, it is essential to transform conventional pulping process into bio-delignification using lignin's enzymes. Increasing the production of ligninase enzymes from white rot fungi as a whole organism has been the centre of interest of several researchers [24].

Nevertheless, enzymes activity created from fungi are relatively slow. The reason of these slow activity can be attributed to the fact that lignin modification is a secondary metabolism which will only be expressed under specific circumstances by the enzymes. Lignin modification have been described by some *Actinomycetes sp*. and soil bacteria such as *Norcadia sp., Rhodococcus sp.* and *Pseudomonas sp.* [24,25]. Nonetheless, Ligninolytic bacteria has not been fully explored and research in this area can lead to several discoveries. Bacterial delignification for biotechnological approach is beneficial over fungi, as the prior is more suitable in molecular genetics and protein expression, efficient in large growth scale and thermo-stability [26].

Pulping is an essential stage in pulp and papermaking technology and can be performed using 3 types of pulping. These classes can be addressed as mechanical, chemical, and thermal [12]. Pulping can also be carried out using a mix of these 3 pulping. Adequate pulps for papermaking are categorized by several factors such as opacity, bulky thickness and light scattering coefficient [27]. Usually, the opacity of the pulps fashioned through mechanical pulping is regarded as good. However, paper fashioned using chemical pulping exhibits higher mechanical paper properties [27].

Mechanical pulping is mostly used due to its lower cost and higher pulp yield factors [9,27]. Even though the yield of mechanical pulp is higher than chemical pulp, the quantity of single fibres is still less as some fibres still incline to be in bundle forms. Mechanical pulp might produce high surface roughness and blending paper could possess low optical properties paper. Increasing the number of refining cycles can improve the surface roughness and applying pre-treatment process could improve the optical properties prior to mechanical pulping process may decrease lignin content in the papers and increase the porosity, fibrillation swelling, and carbohydrate recovery[27].

Mechanical pulping application was addressed as cost-effective method in pulp for paper. Good opacity and high yield of pulps can be obtained using mechanical pulping compare to chemical pulping, that in turn, increase the revenue achieved by producer. Furthermore, mechanical pulping consumes less chemical equated to chemical pulping. Hence, mechanical pulping can be addressed as eco-friendly that produce biodegradable paper and play an important role in reducing global pollution.

2. Materials and Methods

The biomass materials selected for this research were oil palm leaves (OPL), oil palm trunk (OPT), and empty fruit bunch (EFB) were randomly selected from Palm Oil Plantation in Parit Daun, Parit Raja.

2.1 Materials Preparation

The materials were kept in chiller room at 3-4°C in que before proceeding with washing and cutting were done prior to pulping process. The dry leaves of oil palm were cleaned with cloth before cleaning with tap water to remove the dried soil on the leaves. Further-

more, all the samples were washed for about three to four times with tap water and then cleaned through distilled water to remove dirts. The samples were dried overnight at 40°C to remove water moisture.
The fibres then were cut around 2 cm length and kept in boxed or sealed bags at room temperature to prevent fungus growth or any contaminants. All samples were need to be immersed in Erlenmeyer flask of 500 mL with distilled water overnight. The next day the water in the flask was thrown out and the flask were covered with aluminium foil labelled, and then autoclaved at 121°C for 15 minutes before continuing with bio-pulping treatment. The autoclaved samples could only be use 24 hours prior to submerged fermentation to avoid growth of contaminants within the autoclaved samples. The aluminium covers were make sure to be properly sealed on the flask before use.

2.2 Bacteria Treatment (Bio-pulping)

The bacteria used was *Bacillus sp.* isolated from the Termite guts *Coptotermes curvignathus* collected from Universiti Putra Malaysia, Bintulu, Sarawak, Malaysia. The bacteria was cultured for the use of lignin degradation on the samples. Enzymes production was cultivated via submerged fermentation (Erden et al., 2009).

2.1.1 Bacteria Cultures

Firstly, bacteria selected were plated on the agar plate and was kept overnight in the incubator at 37°C. After 24 hours then the bacteria plate was transferred into universal bottle with Luria Broth of about 10 ml autoclaved. The inoculum was left incubated until the solution becomes cloudy or left overnight. Moreover, the suspension of the organism will be determined at a fixed density 0.5 McFarland standard [MF]; 1.5 x 108 CFU/ml and checked via optical density (OD600) measurements using UV-vis spectrophotometry at a minimum reading of 0.8 OD approximately 1 x10^8 cells.
The culture of 1 ml was then transferred into the autoclaved Erlenmeyer flask of 500 mL filled up with the samples and added with Luria broth (LB) medium of about 250 ml autoclaved. The flask was then closed back with aluminium foil and sealed with parafilm tape to avoid any impurities. It is then left in the incubator shaker at pH 6.5 at 35℃, 120 rpm left for 7 days. The negative control was the LB medium and the samples without the bacteria as contamination detector. All plating and transferring media were done in the biosafety cabinet as the bacteria are labelled as class 2 hazardous organism. The samples were weighed and were submerged into the 500 ml Erlenmeyer flask. The weight of each samples were prepared as stated in Table 1.

Table 1: The weight of samples immersed in 250 ml of Luria Broth in the 500 ml of Erlenmeyer flask.

Sample	Mass of Sample (g)
Oil Palm Leaves (OPL)	12
Oil Palm Trunk (OPT)	7.5
Empty Fruit Bunch (EFB)	5

Once the samples were treated for 7 days, the liquid broth with bacteria were transferred for analysis of protein and then the samples were autoclaved. The samples then were washed three times and were oven dried at 40°C until the remaining moisture left to about 5% left or simply dried for storage and could be continued for mechanical pulping. Each samples were prepared for 250 g at minimum for proceeding with the mechanical pulping.

2.3 Mechanical Pulping

Samples treated were soaked overnight before further pulping. All samples were washed separately under running water and then refined using Sprout-Waldron model D2A505 refiner mechanical pulping (RMP) machine to produce the desired pulp under rotor speed pressure of 220 rpm. The refined pulps were screened by using Somerville Screener according to standard TAPPI T 275 with 0.15 mm pore size removing debris. The wet samples then were spin-dried using machine extract (Neng Shin) and was disseminated on a Hobart mixer. The pulps were ready for handsheet making and was stored in chiller at 4°C for further applications.

2.4 Handsheet Production

The laboratory handsheets making was made 60 gsm for 12 sets of paper using semi-automatic sheet machine (British Hand-sheet Machine) according to standard TAPPI T 205 sp-02: Forming Hand-sheets for Physical Tests of Pulp and MS ISO 5269-1:2007: Pulps Preparation of Laboratory Sheets for Physical Testing. The weight of the pulp was determined as in (1) according to moisture content of pulp (%), where MC stands for moisture content calculated by the moisture content device.

$$\boldsymbol{Pulp, g} = \frac{24g\ (o.d) \times 100}{100 - MC} \quad (1)$$

Afterwards, the pulp were put in a disintegrator tank filled with 2000 ml of water. The specimen were left for about 20 minutes at 3000 rpm. The steps followed with the specimen was transferred into a stock divider with 6000 ml of water and left homogenised for about 15 minutes. Afterwards, 2000 ml were collected for the freeness test according to standard TAPPI T 227 om-99: Freeness of Pulp.
Test on correction were performed for two sets during the stock preparation process before proceeding with hand sheets formation by using British handsheet machine followed by drainage time of pulp evaluated in the standard statement of TAPPI T221-cm 99: Drainage Time of Pulp. The handsheet formed where then transferred onto a press sheet machine to remove excess water at 345 kPa for 15 minutes. The handsheet were then stacked on stacked rings with weigh and dried and conditioned at 23 ± 1°C, 50% relative humidity (RH) according to TAPPI T 402 sp-03: Standard Conditioning and Testing Atmospheres for Paper, Board, Pulp Handsheet and Related Products and MS ISO 187: 2001 for 24 hours before proceeding with the characterisation test [16,23]. The flow chart of the process was shown in the figure 3 below.

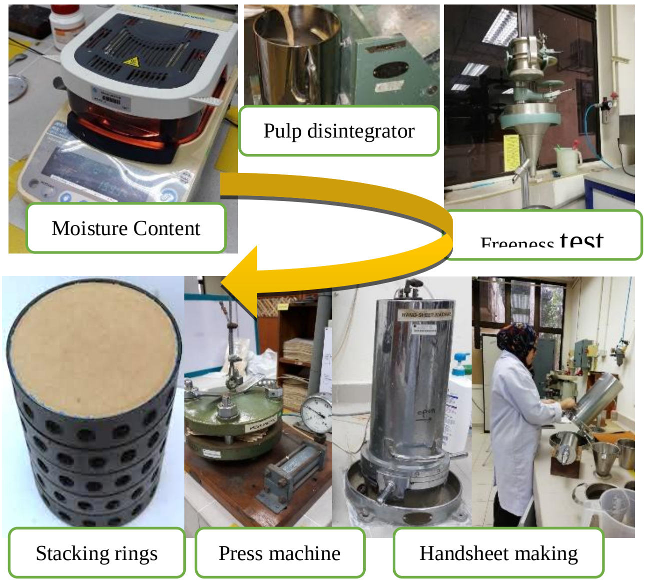

Fig. 3: Flow of handsheet making according to standard TAPPI methods facilitated at Forest Research Institute Malaysia (FRIM).

2.5 Characterization Testing

The characterization of handsheet was conducted on physical, optical and mechanical paper testing. The tests were performed inside a controlled temperature room and humidity condition as required in TAPPI T 402 sp-03: Standard Conditioning and Testing Atmospheres for Paper, Board, Pulp Handsheets and Related Products and MS ISO 187: 200. The physical tests selected in this study were grammage (TAPPI Standard T220 sp-01) while optical paper properties consisted of paper opacity (TAPPI Standard T425 om-01) and brightness (TAPPI Standard T 452 om-02). The mechanical properties were tensile (TAPPI Standard T404 cm-02), burst tested on Frank bursting tester (Frank-PTI) according to (T 403 om-08 TAPPI 2010), thickness test standard TAPPI T 411 om-10, 2010) and tear strength test (TAPPI Standard T414 om-98). The grammage test were done after tearing test on 16 pieces cut were placed into the oven for not less than 30 minutes at a temperature of 105°C.

3 Results and Discussion

The research were conducted on three samples of oil palm biomass: oil palm leaves (OPL), oil palm trunk (OPT), and empty fruit bunch (EFB) prepared in minimum mass of 250 g for hand sheet production. Based on the mass of specimen in previously table 1 showed that EFB biomass needed huge amount for the handsheet production compared with other biomass followed by OPT then OPL. OPL has more mass for the production, however EFB has been reported [16] to have higher biomass production over the years compared with other biomass parts. Thus higher production of biomass could be used for alternative paper based product.
Table 2 shows the characteristics of pulp on freeness and drainage value. Freeness is an important aspect in machine operation where low freeness contribute to low operation [28]. The data showed that all samples treated had a decrease in the freeness value however had a slight increase in the drainage time. The control sample for oil palm leaves had the highest freeness level of 665 ml and the lowest by OPT control sample 650 ml of average. For the treated samples OPT showed to have the highest freeness value while treated EFB was the lowest at 580 ml and performed short drainage time of around 4.02 seconds. However all performance are acceptable as potential paper making as the results decreased in the freeness level and also drainage time that could contribute for lower operational time in paper production but their ability of water resistant would be low. All the fibres absorbed are acceptable amount of water drainage about 300-700 ml which is economical for paper making production [27].

Table 2: Characteristics of pulp on the untreated and treated oil palm Biomass sample

Pulp Properties	Samples of Oil Palm Biomass	
	Control OPL	Treated OPL
Freeness (ml)	665	615
Drainage (s)	4.56	4.07
	Control OPT	Treated OPT
Freeness (ml)	650	630
Drainage (s)	4.35	4.09
	Control EFB	Treated EFB
Freeness (ml)	655	580
Drainage (s)	4.34	4.02

In Table 3, the mechanical testing were done on the 6 type of specimen which resulted the highest grammage tested were treated OPL. Meanwhile, treated EFB has the least grammage weigh since EFB had lighter weight. The low grammage value that is increased for paper making could produce high reading of thickness which contributed to thicker paper and strength [29, 30]. It was found that mechanical pulping performed less sufficient strength on the specimens since all the papers produced were unable to be detected by the tensile test machine except for treated EFB. The control OPL sample were ruptured and could not be produced as handsheet as shown in figure 4 on the comparison with the treatment paper produced.

Fig. 4: The overview of handsheet produced. a) Oil Palm Leaf Control, b) Oil Palm Leaf Treated

This was also due to the fact that EFB has relatively lower lignin content [31] which allowed more inter-bonding compared with OPL and OPT after bacterial treatment. The tensile index for EFB treated was recorded to be 7.144 Nm/g which showed an increament from zero value to the reading of 7. Meanwhile, the tearing index of EFB treated was the highest at 1.685 $Mn.m^2$ /g while OPL recorded to be the lowest at 0.427 $Mn.m^2$ /g. However, the EFB treated thickness was the lowest compared with other samples was due to the lower grammage value thus this have more potential to increase the value strength for higher gsm paper production. All treated samples showed a significant rise in the mechanical strength thus indicated that the bio-pulping of samples improved the strength for paper production for mechanical pulping.

Table 3: Mechanical testing on Hand sheet produced from Oil Palm

Mechanical Tests	Samples of Oil Palm Biomass	
	Control OPL	Treated OPL
Grammage (g/m^2)	56.048	58.487
Thickness (µm)	697.2	713.9
Tensile Index (Nm/g)	-	-
Tear index ($Mn.m^2$ /g)	-	0.427
Burst Index ($kPa.m^2$ /g)	-	0.084
	Control OPT	Treated OPT
Grammage (g/m^2)	56.713	53.609
Thickness (µm)	889.4	770.5
Tensile Index (Nm/g)	-	-
Tear index ($Mn.m^2$ /g)	0.661	0.886
Burst Index ($kPa.m^2$ /g)	0.100	0.208
	Control EFB	Treated EFB
Grammage (g/m^2)	49.516	48.952
Thickness (µm)	717.7	637.3
Tensile Index (Nm/g)	-	7.144
Tear index ($Mn.m^2$ /g)	1.186	1.6850
Burst Index ($kPa.m^2$ /g)	0.146	0.346

The optical analysis was also observed on the opacity and brightness. In table 4, all treated samples showed an increase of ISO opacity n OPL, OPT and EFB around 0.75%, 0.03%, and 1.8% respectively.

Table 4: Optical tests on Hand sheet produced from Oil Palm

Optical Tests	Samples of Oil Palm Biomass	
	Control OPL	Treated OPL
Brightness (%)	13.33	12.37
Opacity (%)	98.70	99.45
	Control OPT	Treated OPT
Brightness (%)	22.57	20.19
Opacity (%)	99.29	99.32
	Control EFB	Treated EFB
Brightness (%)	39.98	33.23
Opacity (%)	97.05	98.80

The increase was relatively small however the effect of treatment did show a percentage increase in all specimen. The ISO brightness however slanted down due to the bacteria treatment before mechanical pulping. The bacteria treatment could possibly form of coloured complexes between the lignin functional groups and metal ions in the water [32,33].The metal ions shows negative effect on the brightness of bleached mechanical pulps compared with unbleached mechanical pulps which generated new functional groups, such as phenolic hydroxyl and carboxyl groups during the treatment process [32,33]. Thus the treatment of the bacteria have the ability in bleaching fibre.

The treated OPL have the best opacity value at 99.45% and the lowest was shown in the EFB control sample at 97.05%, however it has the highest percentage of brightness at 39.98%. Treated EFB did not show a higher opacity since it has a glossy surface shown in figure 5 probably due to the content of oil produced where it could be an alternative for glossy paper production near future. The EFB treated samples had the best improvement results compared with all specimens. In the industry perspective, high brightness and opacity are essential properties of good quality paper enhancing the paper industry's efforts to produce lightweight papers.

Fig. 5: The difference in brightness optical view. a) Oil Palm Trunk Control, b) Oil Palm Trunk Treated, c) Empty Fruit Bunch Control, d) Empty Fruit Bunch Treated

4. Conclusion

The overall result showed that oil palm biomass has potential in paper and pulp production using bio-pulping method. The pre-treatment by bacteria showed clear difference on EFB sample where there was an amount of strength compared with the control and other samples.
The finding shows that, EFB treated samples have lower freeness volume at 580 ml and 4.02 seconds of drainage time which could contribute for lower operational time in paper making. Furthermore, the EFB treated sample also shows better performance in mechanical strength with low grammage value of 48.952 g/m^2, 637.3 µm thickness average, 7.144 Nm⁄g tensile index, 1.6850 Mn.m^2/g tearing index and 0.346 kPa.m^2/g of bursting index.
The result for ISO brightness and capacity, EFB control specimen has the best percentage about 39.98% and 97.05% respectively. Since, the EFB treated samples have the best mechanical strength thus the research found that this sample have the most potential in alternative biomechanical pulping and it also have good brightness and opacity value alongside with the freeness level increased and drainage time decreased.
Further studies on the chemical composition of the untreated and treated samples were needed to analyse the amount of lignin loss due to bacteria treatment. The optical analysis is also will be further analyse using scanning electron microscope (SEM) on the magnification of 100x, 200x and 500x for surface morphology of the handsheet and on its cross sectional view in order to examine the bonding structure of the handsheet production. These would further the investigation and suggesting better optimization of the condition of culturing the bacteria for biopulping is needed.

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