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Lignocellulose chemical composition and handsheet surface morphology analysis on oil palm residue biodelignification treatment using *Bacillus cereus* from *Coptotermus curvignathus*

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Abstract *- Handsheet production in the industrial sector consumed high energy and environmentally unfriendly due to mechanical and chemical methods for the delignification process. In this research, Bacillus cereus isolated from termite (Coptotermes curvignathus) gut's bacteria was used for biodelignification on palm oil biomasses: oil palm leaves (OPL), oil palm trunk (OPT), and empty fruit bunch (EFB). The biodelignification efficiency was analyzed through lignocellulose chemical composition and surface morphology. Lignocelluloses analysis was tested using technical association pulp and paper industry TAPPI T 222 om-02 (lignin content), Kurscher-Hoffner (holocellulose and hemicellulose content), and Chlorite (cellulose content). The highest lignin reduction by Bacillus cereus was 21.7% for treated EFB, followed by OPT (7.0%) and OPL (9.2%). EFB also showed the highest reduction of gap area (76.9%) for scanning electron microscope (SEM) and image analysis with the lowest gap average area (0.03 mm^2) compared with untreated OPL, which were 26.3% and 1.63 mm^2, respectively. Therefore, the EFB handsheet produced showed to be the best potential for industrial commercial.*

Keywords: *oil palm handsheet, biodelignification, lignocellulose, (SEM), empty fruit bunch (EFB).*

I. INTRODUCTION

Virgin pulp is the primary source for pulp and paper production, which annually loses almost 4 billion global trees, comprising 35 percent of all trees harvested in the world [1]. Approximately 91% of the world's pulp and paper is made from wood, mainly in developing countries like China, European countries, and the United States [2]. Countries like Canada, China, Germany, Malaysia, and Russia exported around 45% of global exports (38 million m³) [3]. Additionally, the increased usage of wood in pulp and paper processing has been due to high domestic demand, which raises the concept of interest in finding non-wood plants as alternative fibers [4,5].

Developing countries used around 60% of non-wood raw materials to be produced yearly as an alternative for pulp and papermaking raw material [6,7]. Agricultural residue and annual plant are known to have acceptable lignocellulose properties comparable to wood sources [8]. Previously non-wood materials provided about 10% of the total world production of pulp and paper industries, which covers 44% cereal straws (grains), 14.3% sugarcane bagasse, 14.30% reeds, 21.4% bamboo, and 5.6% others (corn stalks, grasses, and kenaf) [7,9]. China and India are currently known for producing 80% of non-wood pulp since they use around 70% of cereal straw and bagasse [10].

As a future potential for paper making, Malaysia and Indonesia's oil palm biomass has been introduced as oil palm producers [11,12,13]. The idea of oil palm biomass usage happens due to the high quantity of oil palm processed in the industry, which caused waste problems (90%) and also leftovers from the plantation sites (10%) [14]. The production has risen steeply over the years, from about 7.8 million tons in 1995 to approximately 19.5 million tonnes in 2018, as they are readily available in Malaysia [15,16]. The lignocellulose components of oil palm biomass are proposed for commercial paper products [17].

A. Oil Palm Lignocellulose

Lignocellulose is often described as plants' cell walls and the most abundant renewable source on Earth as plant biomass [18]. Lignocellulose components in oil palm biomass are composed of rich cellulose, hemicellulose, and lignin material [19]. Plant polysaccharides mainly comprised cellulose content of about 30 - 50%, lignin 15 - 30%, and the rest of about 20% are accounted for carbon fixation for photosynthesis [20,21].

A significant component of papermaking is cellulose. The amount of cellulose content in a pulp directly influences the pulp's quality by the greater amount of cellulose content [22]. Naturally, lignocelluloses are microbe-degradable and do not release any pollutions and solid waste that might damage the surroundings [23]. Moreover, the various compositions of wood and non-wood fibers (oil palm biomass) are mentioned in Table 1.

Table 1: Wood and non-wood lignocellulose chemical compositions

Biomass	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Hardwood (*Eucalyptus globulus*)[24]	19.9	27.5	53
Softwood (*Pine pinaster*)[24]	26.2	14.1	55.9
Cereal straws[25]	16–18	27–38	44-57
Bagasse[26]	18–24	27–32	32-44
Empty Fruit Bunch (EFB)[4,27,28]	13-37	14.6-33	41.1-65
Oil Palm leaves (OPL) [4,27,28]	18-25	21-24	43-56
Oil Palm trunk (OPT) [4,27,28]	18-23	12-17	29-37

These oil palm biomass samples are ideal for using many polymer composites because of their high cellulose content and high mechanical strength, especially for oil palm EFB fibers [27]. Rushdan and colleges had successfully pulped EFB using an alkaline process [12]. Since then, the subsequent production of Kraft pulp from EFB has been started [29]. However, OPL fibers have potential manufacturing paper, and using an alkaline method for the fiber had produced good paper quality [4]. OPT is not favorable for the particleboard industry than wood sources due to the weak strength of internal bonding. Thus, consideration for the pulp and paper industry is more promising [29].

II. MATERIALS AND METHODS

The non-wood fiber of oil palm biomass substrate selected was empty fruit bunch (EFB), oil palm leaves (OPL), and oil palm trunk (OPT) collected from Parit Daun, Parit Raja, Johor, Malaysia. All samples were washed from the dirt of soil and rinsed three times. Prepared samples were then dried using a universal oven (Memmert, Germany) up to 10% moisture content at 50 °C overnight [30]. Then, the oil palm substrate samples were cut into small chips sizes of about 2-3 cm in length before proceeding with the bio pulping process [31].

A. Biodelignification treatment

Bacteria *Bacillus cereus*, which was isolated from the termite guts (*Coptotermes curvignathus*) collected from Universiti Putra Malaysia in Bintulu, Sarawak, Malaysia, was used as the biodelignification treatment. The bacteria were replicated, plated, and kept overnight at 37°C in an incubator (Incucell, Germany) until it turns cloudy. The suspension was fixed with dilution to a density of 0.5 McFarland standard [MF]; 1.5 x 108 CFU/ml and was confirmed with an optical density (OD600) measured using UV-vis spectrophotometry at a minimum reading of 0.8 OD approximately 1 $x10^8$ cells. It was then cultured and cultivated via submerged fermentation (SmF) [32]. The condition of SmF was adjusted to pH of 6.5, 37°C, and 120 rpm run for 7 days in an incubator shaker using several 500 ml conical flasks covered with aluminum foil and (DaihanSci, Korea) [33].

B. Chemical Composition Analysis

The treated air-dried samples were ground using a grinder mill (Polymix, Swiss) through a 0.40 - 0.45 mm mesh and stored in an airtight container for further analysis. The samples were run for 6 h of extraction process using a Soxhlet using acetone (C_3H_6O) as the solvent extraction at 90 °C. The extraction was done inside a fume hood and followed TAPPI T 264 om-88 for extraction removal. Additionally, the extracted samples were air-dried overnight for further chemical content determination. Samples were kept in a desiccator before and after the weighing process, and samples moisture content was calculated according to the standard method TAPPI T 550 om-08 [34]. The lignocellulose content determination was carried out using standard methods; TAPPI T 222 om-66, Chlorite, and Kurscher-Hoffner, respectively [35]. All the tests were done in triplicate samples.

a) Lignin Content Determination

The lignin content was determined using sulphuric acid (H_2SO_4) according to standard TAPPI T 222 om-02 [36]. Samples of 1 g were measured in a 50 mL beaker and were added with 15 mL of cold 72% sulphuric acid (H_2SO_4) then stirred with a glass rod. The beakers were covered with a watch glass and maintained in a water bath at 20ºC for 2 h. The samples were then continuously stirred until homogenized. Afterward, the homogenized sample was further diluted to 3% sulphuric acid concentration with the addition of 560 mL distilled water in a 1000 mL flask. The flask mixture was then boiled for 4 h and covered to maintain a constant volume in the flask. The mixtures were then filtered using a vacuum suction (GAST, USA) into a Buchner funnel through a Whatman filter paper no. 5. The residue was washed free of acid with 500 mL of hot distilled water. The residue was then oven-dried at 105°C, cooled in the desiccator, and weighed until a constant weight was reached. The lignin content was determined as in Eq. 1.

$$Lignin\ content\ percentage = \frac{W_2}{W_1} \text{ x } 100\ \% \quad (1)$$

Where W_1 is the weight of the sample and W_2 is the weight of oven-dried sample residue.

b) Holocellulose & Hemicellulose Content Determination

The holocellulose determination was conducted to determine overall cellulose content, which was then used to quantify hemicellulose content. This method was carried out using a chlorite method [37]. The samples were weighed 2 g in a 250 mL Erlenmeyer flask, added with 65 mL of hot distilled water, 0.5 mL glacial acetic acid (CH3COOH), and 0.6 g of sodium chlorite ($NaClO_2$) into the mixture. The samples were then maintained in a water bath (Memmert, WNB10, Germany) at 75ºC for 1 hour. Continuously, fresh portions of 0.5 mL acetic acid and 0.6 g of sodium chloride were added after every hour for three times with constant stirring. The mixtures were then left in a room temperature water bath overnight without further reaction reagents. The samples were filtered through a Whatman no. 5 filter paper. The samples were rinsed with cold water of about 500 mL until the color changed from yellow to white. The residue was then oven-dried at 105°C ± 2°C, cooled in the desiccator, and weighed until a constant weight was reached. The holocelullose content was determined as in Eq. 2.

$$Hollocellulose\ content\ percentage = \frac{W_2}{W_1} \text{ x } 100\ \% \quad (2)$$

Where, W_1 is the weight of the sample and W_2 is the weight of oven-dried sample residue.

This procedure was continued by Kurscher-Hoffner method for hemicellulose content analysis [38]. The 2 g of oven-dried sample was prepared and placed in a 250 mL Erlenmeyer flask. A solution of 10 mL of 17.5% sodium hydroxide (NaOH) was added to the flask and maintained in a water bath at 20°C. It was then continuously stirred with a glass rod to react for 2 min until the solution fully immersed the sample. Another 5 mL of 17.5% NaOH solution was added for each 5 min interval two times. The mixture was digested for 30 min and maintained at 20ºC. The distilled water of 33 mL was added to the mixture, mixed thoroughly, and allowed to sit for 1 h. The samples were filtered with Whatman filter paper no. 5 washed with 100 mL of 8.3% NaOH solution, severally rinsed with water. It was then added with 15 mL of 10% acetic acid with released vacuum suction, allowed to rest for 3 min. The samples were vacuumed with 1000 mL of hot distilled water until it was acid-free. The residue was oven-dried at 105°C, cooled in the desiccator, and weighed until a constant weight was reached. The hemicellulose content was calculated as in Eq. 3.

$$Hemicellulose\ content\ percentage = \frac{W_2}{W_1} \text{ x } 100\ \% \quad (3)$$

Where W_1 is the weight of the sample and W_2 is the weight of oven-dried sample residue.

c) Cellulose Content Determination

The cellulose content analysis was carried out according to the Kurscher-Hoffner method [39]. Five grams of the extracted sample was put into a 250 mL Erlenmeyer flask. The sample was treated with 125 mL of alcoholic nitric acid solution (a mixture of 65% w/w nitric acid (HNO_3) solution and 95% purity ethanol (C_2H_6O) at a ratio of 1:4), and the reflux was left for 1 h. The alcoholic nitric acid solution was discarded every 1 h for a total of 4 h, and a fresh portion of the solution was inserted into the flask. The sample was filtered with 1000 mL of hot distilled water until acid-free with Whatman filter paper no. 5. The residue was oven-dried, and the content was determined as in Eq. 4.

$$Cellulose\ content\ percentage = \frac{W_2}{W_1} \text{ x } 100 \quad (4)$$

Where, W_1 is the weight of the sample and W_2 is the weight of oven-dried sample residue.

C. Handsheet Surface Morphology

Handsheet production was carried out at Forest Research Institute Malaysia (FRIM) in the previous experiment according to TAPPI standards [32]. Handsheet samples were cut into different small pieces of about 1 cm, as shown in Fig. 1, and were then subjected to coating. The samples were placed on the specimen stub and then sputter-coated (Quorum Q150R S, UK) with a thin layer of gold-palladium alloy. The surface morphology analysis was evaluated using a scanning electron microscope (SEM) series SU1510 (FUSIO Hitachi, Japan).

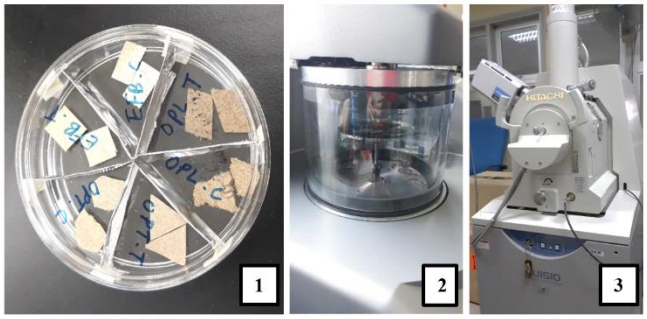


Fig. 1: The following process for scanning electron microscope (SEM) analysis. 1) Preparation of samples 2) Coating and, 3) SEM analysis.

A specimen was placed on a metal holder with the coated layer was put on top, hand handling was avoided. The sample was adjusted to about 13 - 25 mm distance (i.e., the distance between the bottom of the SEM column and specimens' top). The chamber was vented from the first tab for approximately 90 s. The sample was put into the SEM chamber for analysis and was operated at 10 kV [40]. The surface morphology was visualized at 50x and 200x magnification.

III. RESULTS AND DISCUSSIONS

The discovery of *Bacillus cereus* lignin-degrading enzyme extracted from *Coptotermus curvignathus* for its biodelignification application was further studied on handsheet production, which observed the lignin content of oil palm residue reduction and paper strength. However, other aspects of the structure of lignocellulose, such as hemicellulose and cellulose, are also essential in assessing the ability of delignification. In proving the result of lignin reduction, handsheet surface morphology was analyzed to support the lignin content reduction effects.

A. Chemical Composition Analysis

The findings of lignocellulose diversion on OPL, OPT, and EFB residue before and after biodelignification treatment showed a significant difference ($p < 0.05$) for all three chemical contents reduction using ANOVA single factor. The results of chemical composition were shown in Table 3.1. The standard deviation values for the sample oil palm trunk (OPT) are higher, especially in lignin, holocellulose, and hemicellulose material. Meanwhile, the untreated OPT has shown the maximum standard deviation for cellulose.

In general, the highest total proportion of chemical content was contained by the untreated OPL study. This is due to the maximum chemical content observed from an untreated residue. OPL has high lignin content compared with other oil palm residue and high holocellulose and hemicellulose content [4]. Treated EFB residue displayed the lowest chemical composition total percentage at 91.1 % due to the highest decrease in overall contents. This showed higher potential for EFB papermaking with lower chemical content.

a) Lignin Content Analysis

For the oil palm biomass lignin concentration value, untreated palm residues' lignin content was within the range of the literature review reported. It demonstrated a highly likely precise outcome, while different plant growth with different supplemented nutrients induces variation in lignocellulose composition [41]. The selected oil palm residues are acceptable for paper production since all values recorded below 30% in plants have satisfactory pulp and paper-based industries [42].

The results as seen in Table 1 and Fig. 2, empty fruit bunch sample (EFB) had the lowest level of lignin at approximately 17.4% comparative to the oil palm trunk (OPT) sample with 19.3% and 25.2% of oil palm leaves. Moreover, the degree of decline in OPL and OPT indicates an almost equal 2.2% decrease in the gap, with OPT showing a higher reduction in lignin material. Surprisingly, EFB treated pulp reveals a substantial improvement in lignin quality with a decrease of about 21.7%.

Table 1: Lignin content in untreated and treated samples.

Substrate	Lignin Content (%)		Percentage Difference (%)
	Untreated	Treated	
OPL	27.1 ± 0.17*	25.2 ± 0.17*	-7.0
OPT	21.3 ± 0.08*	19.3 ± 0.26*	-9.2
EFB	22.2 ± 0.12*	17.4 ± 0.22*	-21.7

*standard deviation value.
(-) the negative sign indicates a reduction in its percentage value.

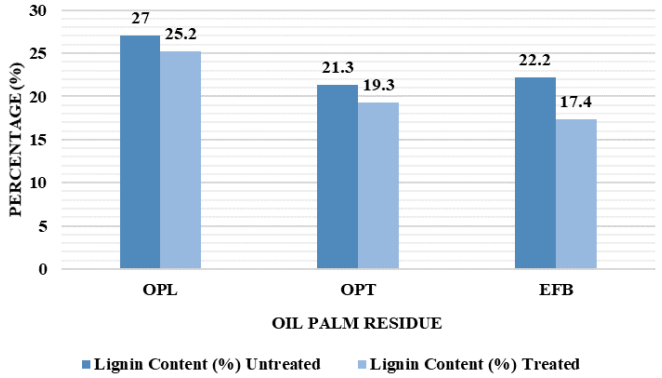


Fig. 2: Graph bars indicated the percentage difference of lignin contents of untreated and treated samples.

This reduction was considered in range, with the commercial fungal degradation extending from 20% to 85% of lignin removal and mineralization [43]. Compared to *Chrysonilia sitophila*, *Bacillus cereus* was higher for about 1.7% and produced in just 7 days for its lignin degradation [43]. Moreover, the presence of lignin degraders from *Bacillus cereus* was firstly sequenced by this study for bacterial lignin degradation while other studies had been concentrated on cellulase.

The reduction of lignin thus leads to a higher quality of high pulp yield production, which the lower lignin content leads to milder pulping conditions (lower temperature and chemical liquor) [42]. The lignin reduction measurement shows the bacteria *Bacillus cereus*'s ability for the lignin removal to produce a high-quality paper. It is suggested that the reduction of lignin in these products has the potential for fast bleaching processes with less chemical liquor use, which ultimately decreases the release of an environmental threat [44].

b) Hemicellulose Content Analysis

The hemicellulose content of the oil palm residue treatment pulp shown in Table 2 and Fig. 3 recorded the highest value for untreated was OPL at 33.95, and the lowest value was 30.0% OPT. The high content value of hemicellulose may have decreased hydrogen bonding's cellulose content for handsheet strength, which produces poor mechanical ability. The OPT treated sample increased but remain to have less content value compared with OPL

and EFB. The treated OPL and EFB samples showed a decrease of approximately 3.5% and 6.45, respectively.

Table 2: Hemicellulose content in untreated and treated samples.

Substrate	Hemicellulose Content (%)		Percentage Difference (%)
	Untreated	Treated	
OPL	33.9 ± 0.21*	32.7 ± 0.16*	-3.5
OPT	30.0 ± 0.18*	30.9 ± 0.54*	3.0
EFB	33.4 ± 0.22*	31.4 ± 0.41*	-6.4

*standard deviation value.
(-) the negative sign indicates a reduction in its percentage value.

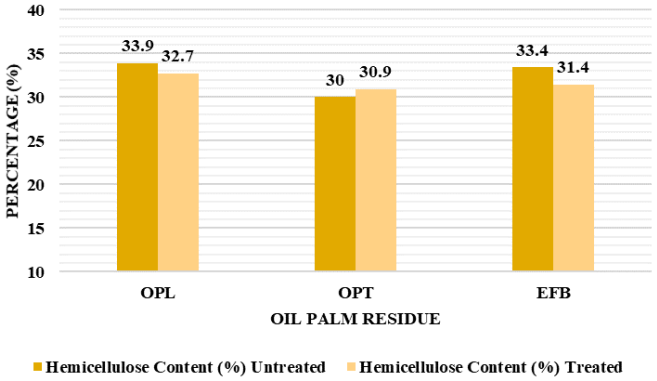


Fig. 3: Graph bars indicated the percentage difference of hemicellulose contents of untreated and treated samples.

The reduction in EFB treated fibers was expected due to high lignin degradation, giving access to the hemicellulose enzyme attack. The reduction of hemicellulose could give more bonding strength between exposed cellulose. However, the reduction in hemicellulose content could give access to cellulose degradation. It is because hemicellulose acts as a wall barrier towards cellulose structures [45]. It also might reduce more cellulose availability for handsheet produced.

However, hemicellulose also contributes significantly to pulp quality (Weiping and Adriaan, 2011) and paper-based products as it promotes pre-grinding fiber swelling (Testova, 2006). Based on the studies, hemicellulose's content value was expected to produce better strength paper quality, especially for tensile, tearing, and bursting [46]. The inclusion of hemicellulose in the cellulosic pulp may boost certain papermaking features where time and energy are used to obtain the required fibrillation level, which may be decreased during the process [47]. Hence, oil palm residues are a good candidate for an alternative fiber.

c) Cellulose Content Analysis

The OPL and EFB samples had a decreased cellulose value content of respectively 0.5% and 8.5%, while OPT has increased just 0.1% based on the findings mentioned in Table 3 and Fig. 4. The higher the cellulose content, the better the quality of paper produced, as in this experiment, OPL should produce the best paper product with 43.6% of cellulose content [22]. The higher the cellulose content amount would have, the better the pulp fibers' bonding to produce a quality paper. Conversely, EFB had the highest degradation of lignin and hemicellulose, and it is hypothesized to have triggered multiple activations of the enzyme that could concurrently degrade the cellulose. Although the importance of lignin loss and hemicellulose loss in the EFB treated sample was found to be the largest, there is a correlation between the strong reduction in cellulose.

Table 3: Cellulose content of untreated and treated samples.

Substrate	Cellulose Content (%)		Percentage Difference (%)
	Untreated	Treated	
OPL	44.0 ± 0.39	43.8 ± 0.36	-0.5
OPT	40.9 ± 0.52	41.0 ± 0.06	0.1
EFB	45.9 ± 0.10	42.3 ± 0.37	-8.5

*standard deviation value.
(-) the negative sign indicates the reduction in its percentage value.

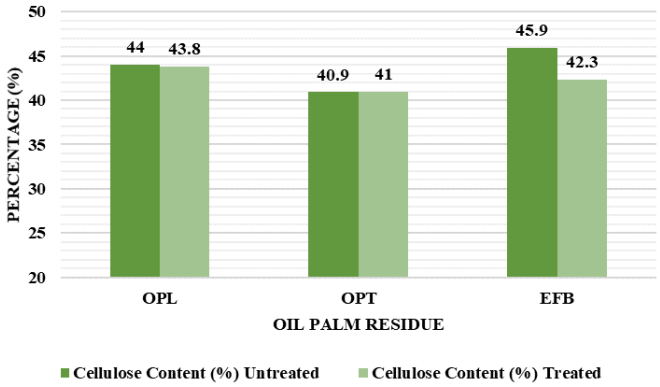


Fig. 4: Graph bars indicated the percentage difference of hemicellulose contents of untreated and treated samples.

Furthermore, previously reported that the bacteria gut of this *Coptotermus curvignathus* could produce cellulytic enzymes [48]. It is possible that in the previous study on both *Trametes versicolour* and *Stereum hirsutum* delignified the chips by 16%. The hemicellulose and cellulose were reduced at 5% for *T. versicolour* and 9% each for *Stereum hirsutum*. As the treatment period increased, both fungi' selectivity values decreased because cellulose was degraded and lignin [49].

Köpcke (2010) reported that the high cellulose content (>34%) in plant materials is characterized as a promising candidate for pulp and paper-based industries [50]. As reported in this study, the amount of cellulose preserved by the oil palm residues remains above 39.6%, which is the lowest value determined in EFB content. Therefore, based on the cellulose content, all of the substrates are acceptable to be used as an alternative fiber in pulp and paper-based industries.

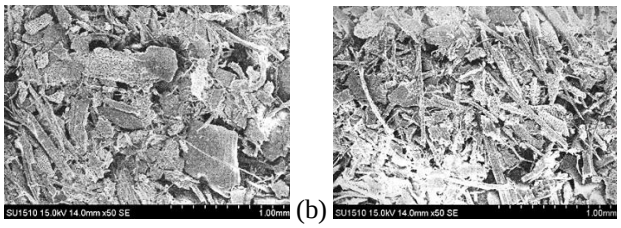
B. Surface Morphology

Scanning Electron Microscopy (SEM) was further used to investigate the morphological structures of each untreated and treated substrates of OPL, OPT, and EFB samples, respectively. The analysis of surface morphology was needed to analyze the fiber bonding structure prevailed by the *Bacillus cereus* treatment, affecting the paper's strength. The SEM images shown in Fig. 5 are the handsheet surface overview structure for 50x magnification. The compactness differences for handsheet untreated and treated of OPL, OPT, and EFB substrates were evaluated by observed concise and equivalent inter bonding structure with minimal gaps area, which shows fiber bonding effectiveness.

Table 4: The average Area of inner bonding gaps for oil palm residue in 200x magnification.

Average Area (mm^2)	Magnification
	50x
OPL Untreated	1.63 ± 1.10*
OPL Treated	1.20 ± 0.64*
OPT Untreated	0.61 ± 0.33*
OPT Treated	0.26 ± 0.21*
EFB Untreated	0.42 ± 0.29*
EFB Treated	0.18 ± 0.19*
*standard deviation value.	

The gaps between inter bonding were analyzed and, as seen in Fig. 5 and recorded in Table 4, were estimated for their average Area. The oil palm substrates display a reduction in gaps between the inter-bonding fibers for the treated samples. This showed a closer attachment structure between the fibers and the *Bacillus cereus* treatment. The gaps observed were for the fiber bonding's outer surface only, but there are further gaps between fibers' layers under the viewed surface. The biggest gap area was recorded by the OPL untreated structure, which is 1.63 mm^2. OPL structure was rough as the handsheet was produced and could not form a firm piece [32]. Meanwhile, the minimum gap area was at about 0.18 mm^2 or equal to 180 μm^2 recorded on the EFB treated sample, which shows the best fiber bonding structure as this will produce better paper strength.



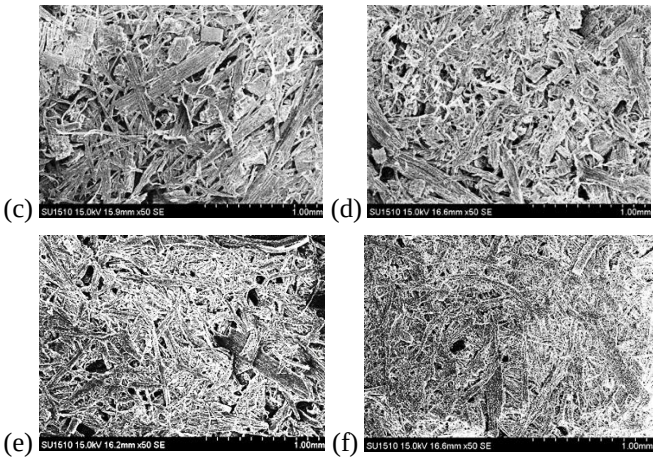
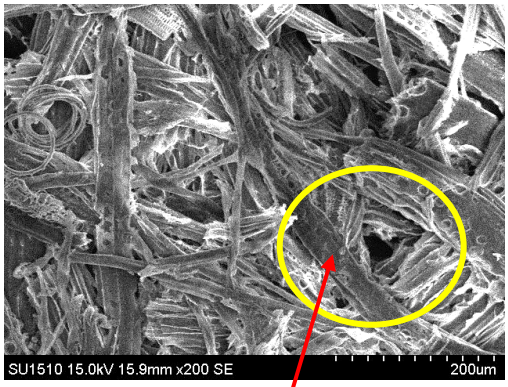


Fig. 5: Surface morphology (SEM) of oil palm residue handsheet images (50x magnification), (a) OPL untreated, (b) OPL treated, (c) OPT untreated, (d) OPT treated, (e) EFB untreated, and (f) EFB treated.

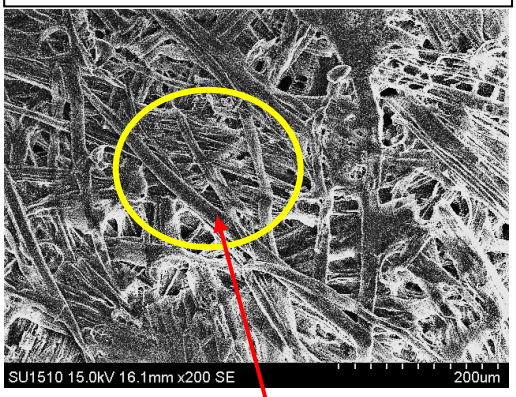
a) Scanning Electron Microscope (SEM) of Empty Fruit Bunch Analysis

In comparison with OPL and OPT, EFB handsheet structure has the best inter bonding structure shown in Fig 5. The surface appearance of the EFB in its fiber inter bonding was even more uniform. As seen in Figure 6, the untreated and treated EFB handsheet showed several cellulose fiber bonding. The treated EFB small strands between cellulose fiber shown to be the hemicellulose bonding. The surface morphology of EFB supported the mechanical studies in which this substrate had the best mechanical strength (48.95 g/m^2 of grammage value, 637.3 µm of thickness, tensile index of 7.144 Nm/g, tear index of 1.69 $mN.m^2/g$, and burst index of 0.346 $kPa.m^2/g$) as well as rigid or firmness structure compared with OPL and OPT handsheets [32].

The handsheet produced for the treated EFB had a compact structure that seemed to provide a smoother surface with improved fiber bonding covering the small pores [51]. Substrate EFB with treatment sample developed stronger hydrogen bonding properties, narrowed gaps, and greater bond regions, resulting in increased stability between contact fibers and improved tensile strength [52]. For a non-wood source with bacteria treatment to develop handsheets, EFB treated handsheets may also be recommended.



a) The fibre bonds were less and uneven. The fibre size was thicker compared with (b).



b) The fibre bonds were visible with less gaps. The EFB structure had really great compactness and more evenly fibre size.

Fig. 6: Empty fruit bunch (EFB) 200x magnification of scanning electron microscope surface morphology, (a) untreated EFB, and (b) treated EFB.

IV. CONCLUSIONS

The *Bacillus cereus* enzyme extracted from *Coptotermus curvignathus* proven to degrade lignin structure and degrade several other lignocellulose content parts. Without enzyme extractions from the bacteria, the EFB substrate performs the degradation of hemicellulose content (6.4%) and cellulose (8.5%). This was analyzed due to the removal of lignin and hemicellulose, which enhance the reduction in cellulose content. Overall, the degradation of lignin affects the fiber bonding of a handsheet formed. The strength of paper is also increased with lower lignin content as hemicellulose and cellulose bonds are more visible in the SEM imaging, which produces the hydrogen bonding. The chemical composition analysis, which complements the surface morphology analysis for treated EFB, shows great potential in paper and pulp making. It is important to create developments in the optimization of biodelignification and additional industrial specifications to ensure improved mechanical strength and the paper's highest quality. The bacterial potential for lignin degradation should be further explored for advanced optimization and understanding of the bacterial enzyme mechanism. Lignin removal materials should produce zero waste industries and implement green technology in future pulp and paper industries.

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