# BIOLOGICAL DELIGNIFICATION OF SHREDDED OIL PALM EMPTY FRUIT BUNCH USING MYCELIA CULTURE OF *GANODERMA LUCIDUM* AS A POTENTIAL RUMINANT FEEDSTUFF

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**ABSTRACT**

*Livestock production in Malaysia is still inadequate to meet the demand especially ruminant products. Efforts were made to increase the availability of local feed by having a better utilization of agro-industrial by-products which can increase the production of ruminant products. The extraction of palm oil produced abundant of empty fruit bunch (EFB), which considered as waste and has raised environmental concerns. It is a lignocellulosic raw material which consist high cellulose, hemicellulose and lignin. Researchers has reported that white-rot fungi have the ability for delignification by extracellular enzymes Thus, this study evaluated the cell wall constituents (cellulose, hemicellulose and lignin), the morphological surface changes, and in-vitro dry matter digestibility (IVDMD) of oil palm EFB fibres treated with mycelia culture of Ganoderma lucidum. Shredded EFB fibres were obtained and prepared as sterilized substrate in polypropylene bags, inoculated with mycelial culture of G. lucidum and incubated for 4, 8 and 12 weeks of incubation period. Result showed that there was significant reduction on EFB fibres fibre composition after treated with G. lucidum until reach the lowest at 12 weeks of incubation where 27.77% to 22.08% for cellulose, 19.30% to 14.15% for hemicellulose, while 12.69% to 7.49% for lignin content. The SEM images showed that silica bodies were removed from the strand surface and left only exposed pores at the bottom after treatment with G. lucidum. The IVDMD of treated EFB fibres were increased as incubation period increased where the highest at week 12 of incubation with 81.88%. These results suggested that the G. lucidum was effective in delignification of EFB fibres after 12 weeks of incubation and have the potential to be utilized as ruminant feedstuff. Utilization of biomass generated from palm oil mill not only will solve our environmental problem but also convert low quality biomass into valuable local feed for ruminants.*

Keywords: Ruminant feed, feed composition, oil palm by-product, empty fruit bunch, Ganoderma lucidum..

## INTRODUCTION

Malaysia is one of world's largest palm oil producers and exporters, where Malaysia produces approximately 18.86 million metric tonnes of palm oil, which contributed to 30% of the global palm oil supply (USDA, 2018). The palm oil industry in Malaysia generates huge quantities of biomass in the form of oil palm empty fruit bunch (EFB), oil palm shells (OPS) and oil palm fibres (OPF). The potentials of these biomasses are yet to be fully exploited as it currently leads to waste disposal problems (Yaakob *et al.*, 2012).

About 20% of oil palm EFB is produced from the processing of a fresh fruit bunch. Oil palm EFB fibres has low economic value and due to its large quantity of production, has risen many environmental concerns. Conventionally, EFB waste is usually burned, disposed of in landfills or composted to become organic fertilizers in oil palm plantation. However, burning of EFB wastes is not recommended and has been banned as it causes air pollution and other environmental problems. It is therefore, important to optimally utilize the EFB waste from palm oil mill in order to solve these problems and at the same time, utilizing available resources for valuable products (Isroi *et al.*, 2012).

In the meantime, livestock industry is also one of Malaysia's important industry as it contributes to agricultural development. Even though ruminant sub-sector showed progress in recent years, however, its self-sufficiency level (SSL) is still less than 30% (beef 23.50%, mutton 11.46%) (Rozhan Abu Dardak, 2019). This means that the ruminant products from local sources are still inadequate to meet the local demand as following the increase in population and consumption (DVS, 2014). In National Agro-food Policy 2011-2020 (NAP4), the demand is projected to increase from 1.4 million MT in 2010 to 1.8 million MT in 2020. One of the major reason for the slow growth of the ruminant industry in Malaysia is the lack of good-quality feed resources. That is why, there is need to fully utilize and have a great dependent on agro-industrial by-products especially from oil palm industry for ruminant feed resources

The widespread use of EFB waste as livestock feed is still constrained by its low digestibility. It is one of a lignocellulosic raw material, where lignocellulose is a major part of a plant cell wall, which consist of carbohydrates namely, cellulose (37-59.7%), hemicellulose (16-28%) and lignin (10-32%) (Abdullah & Sulaiman, 2013; Baharuddin *et al.*, 2013; Sudiyani *et al*., 2013). That is why, an alternative approach has been proposed to improve degradability of EFB fibres or any possible agricultural by-products for ruminant feeding which is by using lignocellulolytic fungi (Nur Nazratul *et al.*, 2019).

Researchers has reported that the lignocellulose material can be treated by biological processes, which is by using microorganisms mainly white-rot fungi (Taniguchi *et al*., 2005). These white-rot fungi produce ligninolytic enzymes, that can degrade lignin efficiently (Isroi *et al*., 2011). These fungi also degrade holocellulose and decrease the crystallinity of cellulose. Biological pre-treatment has the potential to be developed and applied industrially, as it has a few advantages for instances, low energy requirement, low capital investment, no requirement for chemicals, mild environmental conditions and simple process and equipment requirements. Thus, the objectives of this study were to evaluate the cell wall constituents (cellulose, hemicellulose and lignin), the morphological surface changes, and in-vitro dry matter digestibility (IVDMD) of oil palm EFB fibres treated with mycelia culture of *Ganoderma lucidum*.

## MATERIALS AND METHODS

Lingzhi mushroom (*Ganoderma lucidum*) sample was obtained from a local mushroom farm in Penampang, Sabah. Its pure culture was isolated by standard tissue culture technique from the basidiocarp (Scrase, 1995) using potato dextrose agar (PDA) media. Slant stock culture was then prepared and maintained at 4 $^{o}$C in PDA for further use. Working culture prepared by sub-culture technique and incubated for 7 days in 25-28 $^{o}$C in the dark before inoculated with sterilized EFB fibres substrate.

Shredded oil palm EFB fibres with an average length of 5 cm were obtained from a local palm oil mill which located in Lahad Datu, Sabah. The sample was freshly collected and sun-dried (to less than 10 wt. % moisture content) until further use to reduce its moisture content and to prevent the growth of moulds. After sun-dried, the EFB fibres were weighed (100 g each) into trays and soaked in distilled water overnight to allow them to absorb water. Then, the EFB fibres were toasted and inserted into polypropylene bags. All bags of EFB substrate were then sterilized using autoclave machine (Tomy, SX-700) at 121 $^{o}$C with 15 p.s.i. for 15 min. The autoclaved EFB substrate were left to cool to room temperature (25-28 $^{o}$C) before inoculated with *G. lucidum* mycelia culture.

The sterilized EFB substrates were then inoculated with 16 plugs (5 mm) of 7-day old *G. lucidum* mycelia while other four bags of uninoculated EFB substrates were separated and served as control. All inoculated EFB substrates were incubated at 4, 8 and 12 weeks of incubation period (Table 1). The experiment was conducted in four replications, which made a total of 12 experimental units excluding the control. All experimental units were arranged in completely randomized design (CRD) in room conditions at 25-28 $^{o}$C with 80-85% relative humidity stored in the dark.

After each incubation period, all bags of treated EFB substrates were harvested for sampling. All four replicates from each treatment were homogenized and oven-dried at 70 $^{o}$C until it reached its constant weight. The dried samples were cut into smaller sections (1-5 mm) and kept in sealed bags until further analysis. The same procedure also performed to the uninoculated EFB fibres samples (control).

**Table 1: Experimental treatments.**

| Sample | Incubation period (week) |
|---|---|
| Oil palm EFB[1] fibres only (Sterilized) - control | 0 |
| Oil palm EFB fibres (Sterilized) + mycelial culture | 4 |
| Oil palm EFB fibres (Sterilized) + mycelial culture | 8 |
| Oil palm EFB fibres (Sterilized) + mycelial culture | 12 |

[1]EFB: Empty fruit bunch

All replicate samples as shown in Table 1 were analysed in triplicate for fibre composition namely, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) following the method described by Van Soest *et al.* (1991). The hemicellulose content was calculated as the difference between NDF and ADF. Cellulose content was calculated as the difference between ADF and ADL.

Both dried samples of uninoculated and treated EFB fibres was observed under scanning electron microscope (SEM) by using SEM model MA10 (Carl ZEISS). As preparation, samples were coated with a thin layer of gold by using sputter coater model K550K (Emitech) (Zahari *et al.*, 2018).

*In-vitro* gas production technique was carried out using the method described by Menke and Steingass (1988). The rumen fluid was used as inoculum and collected into pre-warmed thermos bottles before the morning feeding from a fistulated cow which fed mixture of fresh grasses and concentrate. Then, the rumen fluid was filtered using cheese cloths. The filtered rumen fluid then mixed with buffer and mineral solutions prepared under continuous flushing with $CO_2$.

Approximately 0.2 g DM of samples (1 mm) were weighed into syringes fitted with plungers. Buffered rumen fluid (30 mL) was pipetted into each syringes which containing the feed samples and the syringes were immediately placed into water bath at 39 $^oC$ and shaken at regular times. Incubation was done in triplicates. Three syringes with only buffered rumen fluid were incubated and considered as blanks.

All sample replicates were terminated at 48 hours of incubation period for the determination of *in-vitro* dry matter digestibility (IVDMD). The residues were filtered by using sintered glass crucibles and oven-dried at 105 $^oC$ for 24 hours to estimate the dry matter disappearance. The IVDMD was calculated by using the formula below:

$$\text{IVDMD} = \frac{\text{Initial sample weight (g)} - \text{dried residue of sample}}{\text{Initial sample weight (g)}} \times 100$$

All data obtained were analysed by using one-way analysis of variance (ANOVA). Their means were then compared by using the Duncan's multiple range test (DMRT) at significance level of 5% ($p<0.05$). All statistical analyses were performed using the Statistical Analysis System (SAS) 9.4 (2007).

**RESULTS AND DISCUSSION**

After 2 weeks of incubation, it can be observed that the mycelia culture of *G. lucidum* already fully colonized all bags of substrate and the mycelia density was increased until the last of incubation period. Kenealy & Dietrich (2004) has mentioned that a good colonization of mycelia means effective fungal treatment. While Gao *et al.* (1993) reported that mushroom strains have a short cultivation period because they have high levels of enzyme activity.

Fibre composition (cellulose, hemicellulose and lignin) of both uninoculated and treated EFB fibres with *G. lucidum* is presented in Table 2. Based on the results obtained, the fibre composition of uninoculated EFB fibres were in range with findings by Kamcharoen *at al.* (2014) and Husin *et al.* (2002). However, in contrast with that reported by Hamzah *et al.* (2016). Therefore, this means that different sources of EFB fibres from different palm oil mill might resulted in very different fibre composition depending on the mill ways of handling.

It can be observed from Table 2, that all fibre composition of treated EFB fibres were significantly reduced after treated with *G. lucidum*. The cellulose content of EFB fibres from 27.77% (control) were significantly reduced on week 8 (26.17%) and reach the lowest at week 12 (22.08%) of incubation. The hemicellulose content also was significantly reduced starting from week 8 (15.10%) and reach 14.15% on week 12 of incubation. Meanwhile, the lignin content started to reduced significantly from week 4 (10.05%) and reach the lowest on week 12 (7.49%). Generally, fungi require carbon, nitrogen and inorganic compound as their nutritional sources. The main nutrient are carbon sources such as cellulose, hemicellulose and lignin (Tabi *et al*., 2008). Bartnicki-Garcia *et al.* (2000) has mentioned that carbohydrates are important for the fungi especially for its growth as the the cell wall synthesis depends on the glucose supply. Basically, this explains generally the fibre composition of EFB fibres significantly reduced across incubation as being used up by the mycelia of *G. lucidum*.

Lignin was the most recalcitrant to degradation and it is linked to both hemicellulose and cellulose by forming a physical seal, which making it as impenetrable barrier that prevents the penetration or release of other components (Howard *et al.*, 2003). Lignin also was known cannot be degraded through anaerobic fermentation in the rumen and reported negatively related to digestibility in ruminants (Lara *et al.*, 2003). After delignification by *G. lucidum* mycelia culture in EFB fibres, cellulose and hemicellulose present would be useful carbohydrate for ruminants.

**Table 2: Fibre composition of uninoculated and treated EFB fibres with *G. lucidum* at different incubation period**

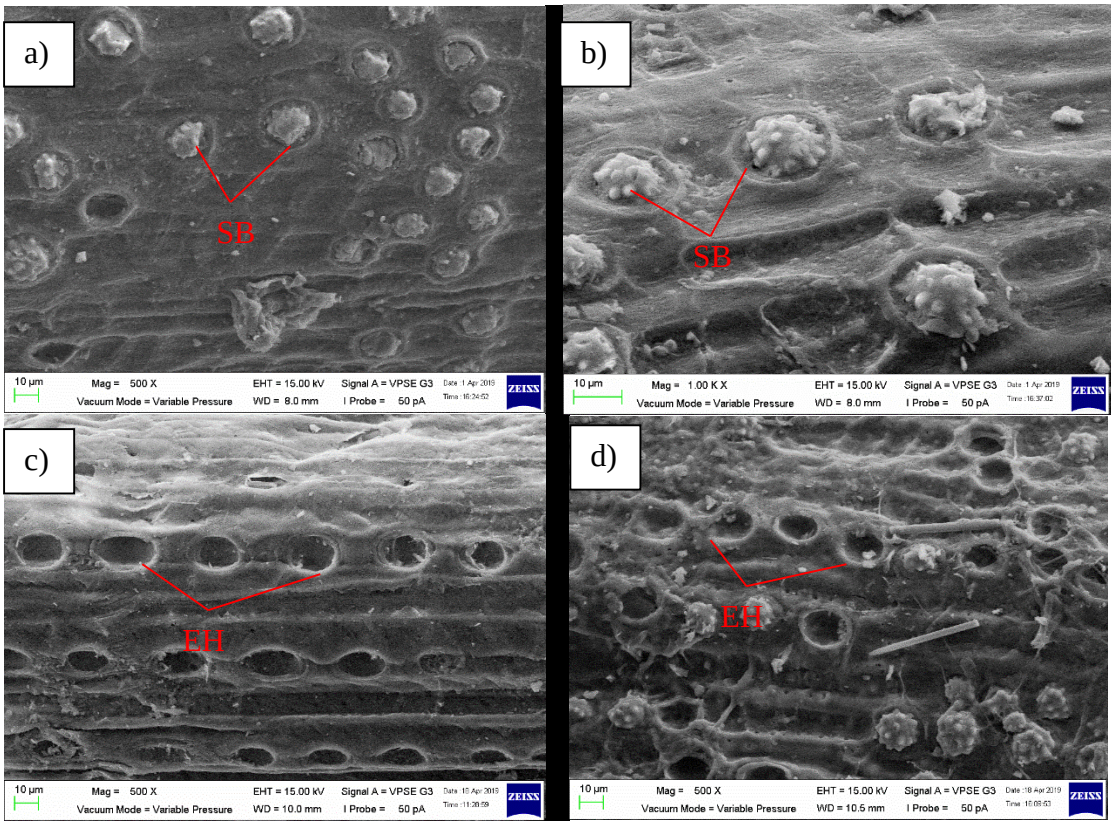
| Composition (%) | Incubation period (week) Mean ± SE | | | |
|---|---|---|---|---|
| | 0 (Uninoculated) | 4 | 8 | 12 |
| Cellulose | $27.77^{ab} \pm 0.67$ | $28.26^{a} \pm 0.61$ | $26.17^{b} \pm 0.67$ | $22.08^{c} \pm 0.28$ |
| Hemicellulose | $19.30^{a} \pm 1.05$ | $18.72^{a} \pm 1.29$ | $15.10^{b} \pm 0.56$ | $14.15^{b} \pm 0.34$ |
| Lignin | $12.69^{a} \pm 0.27$ | $10.05^{b} \pm 0.20$ | $8.58^{c} \pm 0.45$ | $7.49^{d} \pm 0.32$ |

$^{a,b,c}$ Means with different superscript within row differ significantly ($p<0.05$) using Duncan's multiple range test (DMRT). SE: Standard error.
Source: Nur Nazratul *et al.* (2019)

The SEM images in Figure 1 presenting the surface morphology of uninoculated and treated EFB fibres with mycelia culture of *G. lucidum*. Based on the images shown, it can be observed that the uninoculated EFB fibres (Figure 1 a-b) have this slightly smooth with large number of spiky silica-bodies embedded on the surface. The finding was similar with Kamcharoen *et al.* (2014). Those silica bodies were in round-shape with spikes and attached uniformly around the fibres surface. Nascimento *et al.* (2012) has mentioned that silica bodies present on fibre surface provide strength as those silica bodies restrict the sliding motion between the fires. The fibre structure also looks quiet rigid and solid as the surface was covered by a layer of matrix material like lignin or waxes (Zahari *et al.*, 2018).

Based on Figure 1 c-d, it can be observed that after treated with *G. lucidum* mycelia culture, the silica bodies which present on EFB fibres were removed and left only expose pores at the bottom. This was in agreement with finding by Isroi *et al.* (2012) which also study the structural changes of EFB fibres after fungal treatment. Hamzah *et al.* (2011) has mentioned that any fibres without silica bodies can be easily decomposed as the increase in surface area after silica bodies removal which then allow enzyme attack by microorganism to become more active. Law *et al.* (2007) also mentioned in his report that the removal of silica bodies would enhance any penetration such as chemicals or enzymes on the fibre surface. Therefore, treated EFB fibres with mycelia culture of *G. lucidum* as animal feed will have higher surface area and higher digestibility when consumed by ruminants because it can be easily 'attack' by the microorganisms which presents in the rumen.

**Figure 1: Surface morphology of uninoculated and treated EFB fibres with *G. lucidum*. a) Uninoculated EFB (500x magnification), b) uninoculated EFB (1000x magnification), c) treated EFB (500x magnification) and d) treated EFB (1000x magnification). SB: Silica bodies, EH: Empty holes.**




Based on Table 3, it presents the *in-vitro* dry matter digestibility (IVDMD) of uninoculated and treated EFB fibres with mycelia culture of *G. lucidum*. It can be observed that the IVDMD of EFB fibres were significantly increased across incubation period from 46.51% (control) to the highest in week 12 (81.88%). Digestibility is an important factor in the measure of nutrition value of animal feed. Dry matter digestibility are very important determinants for evaluate the nutrients absorbed by ruminants (Al-Arif *et al.*, 2017). Therefore, regarding the digestibility measured of treated EFB fibres, it would be a good quality roughage for ruminants.

**Table 3: Dry matter digestibility of uninoculated and treated EFB fibres with *G. lucidum* at different incubation period.**

| Incubation period (week) | DMD %<br>(Mean ± SE) |
|---|---|
| 0<br>(Uninoculated) | $46.51^{d} \pm 1.32$ |
| 4 | $60.97^{c} \pm 0.55$ |
| 8 | $70.93^{b} \pm 0.46$ |
| 12 | $81.88^{a} \pm 0.88$ |

$^{a,b,c,d}$ Means with different superscript within column differ significantly ($p<0.05$) using Duncan's multiple range test (DMRT). SE: Standard error.

## CONCLUSION

It can be concluded that biological delignification of oil palm EFB fibres by using mycelia culture of *G. lucidum* was efficient and making it potential as ruminant feedstuff. All fibre composition of treated EFB fibres were significantly reduced especially the lignin content which the most recalcitrant component among others. The SEM images of treated EFB fibres also supported the result by showing that the silica bodies and lignin material which covers the fibre surface were removed. Biological delignification by using *G. lucidum* mycelia culture also significantly increase the IVDMD of EFB fibres. It was found that the lignin content started to significantly reduced on week 4 of mycelia culture inoculation, which suggest that 4 weeks of incubation is sufficient to reduce the lignin content of EFB. Furthermore, 12 weeks of incubation is recommended for lower content of all cell wall constituents (cellulose, hemicellulose and lignin) also with the highest digestibility of EFB fibres. Treated EFB fibres indeed has potential as ruminant feedstuff. Utilization of biomass generated from palm oil mill not only will solve our environmental problem but also convert low quality biomass into valuable local feed for ruminants. This in line with NAP4 strategies which to have a sustainable agriculture and developing agriculture especially in livestock sector.

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