

Thermal Degradation and Morphological Changes of Oil Palm Empty Fruit Bunch Vermicompost

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Vermicompost produced from oil palm empty fruit bunch (EFB) was analysed to examine its thermal degradation and morphological appearance. The thermal degradation of vermicompost produced from untreated EFB and EFB treated with oyster mushrooms was characterised *via* thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTGA). It was observed that vermicomposting accelerated the thermal degradation of EFB and minimized the lignin content, but reduced lignin degradation of raw EFB. However, the thermal degradation of lignin increased in treated EFB vermicompost. The structural characterization of EFB vermicomposting revealed that the surface of the treated EFB was more fragmented than the untreated EFB vermicompost and raw EFB.

Keywords: Empty fruit bunch; Vermicomposting; Thermal degradation

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INTRODUCTION

Huge quantities of oil palm empty fruit bunch (EFB) are readily available in the form of oil palm by-product in palm oil producing countries. In particular, EFB is the residual fruit bunch generated after fruits are removed from the fresh fruit bunch (FFB). Malaysia is the world's second largest crude palm oil producer and largest palm oil products exporter (MPOB 2012). It has been reported that about 23 million tonnes of EFB are generated in Malaysia per annum. However, EFB generation would increase with increasing crude palm oil production and a resultant increase in the number of palm oil mills created to meet the rising palm oil demand. Thus, it is necessary to define ecological and economical approaches for the effective utilization of EFB. Studies have been conducted on the utilization of oil palm biomass as an alternative fuel for electricity and steam generation to be used internally in palm oil mills (Chang 2014; Hosseini and Wahid 2014). However, it has been reported that the EFB is not suitable for fuel production due to its high moisture content, which is about 65 wt% (Samiran *et al.* 2016). In the past, EFB has been disposed of *via* small-scale incinerators used to generate steam in mills and utilized in soil mulching (Awalludin *et al.* 2015). The incineration of EFB was banned in 2000 by the "Malaysian EQA 1974 Clean Air Regulation of 2000" due to its high moisture content and tendency to release contaminants in emissions (Yacob *et al.* 2005). The utilisation of EFB for soil mulching has become unpopular due to its phenolic content (Kavitha *et al.* 2013).

Empty fruit bunch contains high cellulose content (40% to 60%) and hemicellulose content (20% to 30%), which has good potential to be used to produce bio-compost

(Siddiquee *et al.* 2017). Generally, the chemical composition of EFB consists of cellulose, hemicellulose, and lignin (Ching and Ng 2014; Siddiquee *et al.* 2017). The biodegradation of EFB is difficult due to the presence of lignin in EFB (10% to 20%). Lignin is resistant to microbial attack and therefore does not easily decompose naturally, requiring further action for decomposition to occur (Hayawin *et al.* 2011). However, numerous studies have been conducted to explore the possible biodegradation of lignocellulosic materials (Iqbal *et al.* 2013; Fatah *et al.* 2014; Abdul Khalil *et al.* 2016; Moya *et al.* 2016). The studies reported that the vermicomposting process is an effective, sustainable process for the biodegradation of lignocellulosic biomass (Sing *et al.* 2011; Iqbal *et al.* 2013). Vermicomposting is a process of bio-oxidation and stabilisation of organic material that, in contrast to composting, involves the actions of both earthworms and microorganisms and does not involve a thermophilic stage (Mengistu *et al.* 2018). Vermicomposting is fundamentally the consumption of organic material by earthworms, which enhances the rate of decomposition and forms a nutrient rich and microbial active end product (vermicompost or worm cast). Gong *et al.* (2017) reported that vermicomposting is the joint action of earthworms and mesophilic aerobic bacteria. During this process, earthworms actively break up the waste substrate, accelerate the rate of decomposition of organic matter, and alter the physical and chemical properties of the material, leading to a reduction in waste volume and the production of a stable compost product (Gong *et al.* 2017; Siddiquee *et al.* 2017). Vermicomposts are finely divided peat-like materials with high porosity, high aeration, high drainage, and high water holding capacity. A vermicompost contains many essential nutrients such as nitrates, phosphates, exchangeable calcium, and soluble potassium in plant available forms (Lim *et al.* 2015). In addition, it has a large surface area that provides sites for microbial activity and retention of nutrients (Kaviraj and Sharma 2003).

Hayawin *et al.* (2011) mixed shredded EFB with cow manure to increase microbial content and accelerate the digestion of substrate by earthworms. Sabrina *et al.* (2009) found that only *Eisenia fetida* was able to survive in EFB pre-composted for one month with palm oil mill effluent (POME) and the addition of cow manure reduced the mortality rate and maintained the earthworm weight in the EFB culture. Vermicomposting enhanced the compost quality with respect to nutrient content (Sabrina *et al.* 2009; Hayawin *et al.* 2010). Sabrina *et al.* (2009) studied the effect of the direct composting of EFB and palm fronds via onsite earthworms in an oil palm plantation and found that *Pontoscolex corethrurus* and *Amyntas rodoricensis* died immediately when added to the EFB. The death of both species was attributed to the phenolic compounds of EFB. However, Ahmad Yahaya *et al.* (2017) observed that the amount of phenolic compounds sharply decreased in treated EFB vermicomposting using *Pleurotus sajor-caju* after five weeks of composting.

Vermicomposting is a simple, feasible, economical, and eco-biotechnological process for managing agro-industrial biomass in agricultural applications (Sabrina *et al.* 2011; Singh *et al.* 2011). Generally, the vermicomposting process transforms the complex organic substances of biomass into stabilized humus-like product, called vermicompost. During the vermicomposting of biomass, earthworms digest biomass substances and leave stable mature materials (Singh *et al.* 2011; Moya *et al.* 2016). The maturing process of the compost involves several changes in the chemical composition and structural transformation of the compost. Thermal analysis is an effective analytical means to determine the organic matter generated during the vermicomposting process. Mendez *et al.* (2011) reported that thermogravimetric analysis (TGA) is an effective analytical tool to

evaluate carbon mineralization and organic matter transformations. Although studies have been conducted on the thermal analyses of compost materials, there are still very few reports on the thermal analyses on vermicomposting. Therefore, the present study was conducted to perform thermal degradation analyses of EFB vermicompost using TGA. The morphological appearance changes of the vermicomposting were also evaluated.

EXPERIMENTAL

Materials

All EFBs were originally collected in the form of short and long fibres from Sabutek Sdn. Bhd (Kuala Lumpur, Malaysia). Plant health movement documents and plant passports (Phytosanitary certificates) were produced by the Malaysian government before shipment to the UK, as required by European Union Legislation and the Food and Agriculture Organization (FAO) International Plant Protection Conventions. The EFBs were ground to sizes of 5 mm to 10 mm using a Glen Creston Beater mill (Retsch GmbH, Haan, Germany), soaked for 2 weeks, and drained well before use.

Preparation of mushroom spent EFB substrate

A pure strain of oyster mushroom *Pleurotus sajor-caju* (DSM 5339) was obtained from DSMZ, Braunschweig, Germany. The delivered strains were a form of mycelia that was cultivated on potato dextrose agar to produce more mycelium for spawning. Mature mycelium on the petri dishes was cut into small pieces using a sterile stainless steel scalpel. Half of the mycelium in a single petri dish was added into the wheat grain in each 300-mL beaker. The beakers were covered with paraffin film and shaken thoroughly. Two petri dishes of mycelium were cut and added into the wheat grain in each 3-L autoclaved bag. The bags were sealed and the mixture of wheat grain and mycelium was shaken thoroughly. Both the beakers and bags were covered with aluminum foil and incubated at 25 °C for 12 days.

When the mycelium covered most of the surface of the wheat, the mycelium was inoculated onto the EFB substrate with the layer spawning method described by Marino *et al.* (2003). Every 10 cm of EFB was covered by a 1 cm layer of spawn grains. A total of 5 wt.% spawn grains of *P. sajor-caju* was used for preparing 5 kg of EFB substrate. The EFB substrate prepared was left in the propagator at 21 °C to 25 °C and a relative humidity of 80% to 90% for 12 h with the propagator windows opened for air ventilation. The fluorescent lights were covered with a blue film to produce a blue spectrum of 440 nm to 495 nm (Stamets and Chilton 1983). The cultivated EFB with oyster mushroom *P. sajor-caju* was referred to as pretreated EFB in this study.

Vermicomposting of untreated and pretreated EFB

A commercial vermicomposting reactor (Worm Work from Original Organics Ltd., Hitchin, UK) was used for vermicomposting, as presented in Fig. 1. The vermicomposting reactors used in this study were enabled for leachate collection and temperature control (aerated). Each reactor consisted of a stack of 4 trays (14 in × 14 in × 5 in). A 0.5 cm × 0.5 cm cotton sieve lining was placed in the base of each tray. Air was pumped into the vermicomposting reactor using two 6-inch fish tank aerator stones that lay on the bottom tray. A sieving fabric with 1-mm diameter poles was placed over the aeration stones and

the base of the tray to prevent young earthworms from passing through the bottom layer of the tray and drowning in the leachate collection. A digital thermometer was used to monitor the temperature in all of the vermicomposting reactors (Fig. 1a). The temperature sensor was placed in the second tray in each reactor.

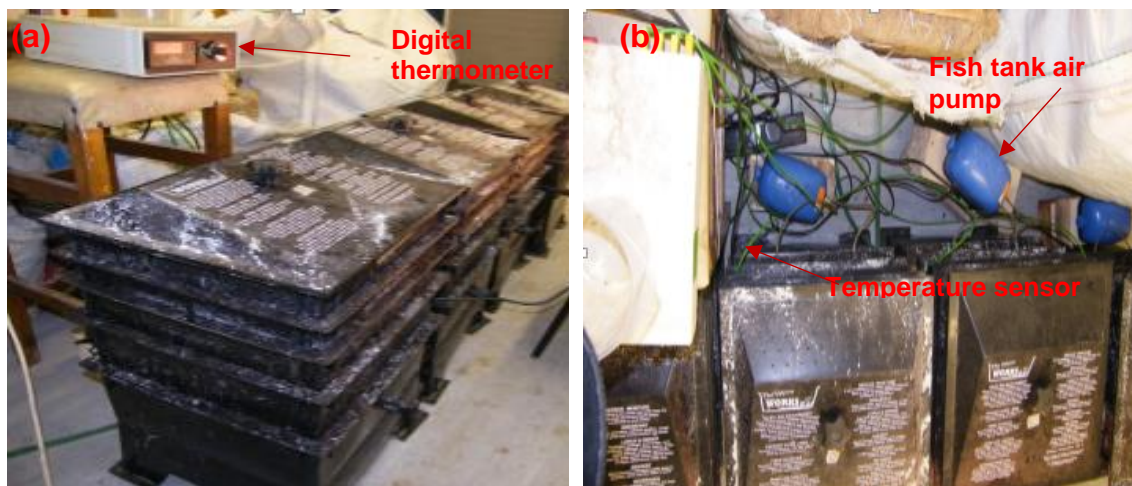


Fig. 1. (a) Vermicomposting reactor, (b) experimental set up of the vermicomposting reactor

Approximately 312.5 g of a mixed stock of juvenile *E. fetida* and mature *E. fetida* were placed in each tray with approximately 1.25 kg of treated EFB and untreated EFB (in 0.109 m² tray area). The stocking density for each tray was 2.87 kg/m², which was within the stocking density range suggested by Frederickson *et al.* (1997). The vermicomposting of untreated EFB and mushroom spent EFB substrate were conducted following the large-scale vermicomposting trials (LSVT). Four replicates were conducted (LSVT1, LSVT2, LSVT3, and LSVT4) for 30 weeks (Frederickson *et al.* 1997).

Methods

Determination of the moisture content

The percentage moisture contents in raw EFB, pretreated EFB vermicompost, and untreated EFB vermicompost were determined following the British standard method for “Soil improvers and growing media” (Standard No. BS EN 13040:2000). About 1 g of ground EFB was added to the pre-weight crucible and the weight was recorded. Then, the crucible with ground EFB was placed in preheated oven at 105 ± 5 °C for 2 h as a drying process. The crucible was let to cool to room temperature and reweighed. The moisture content was then calculated using following equation,

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

where W_1 is the weight of ground EFB and EFB vermicompost and W_2 is the weight of ground EFB and EFB vermicompost after pyrolysis.

Thermogravimetric analysis

The percentage degradation of hemicellulose (hemicellulose and cellulose) and the lignin content in raw EFB, pretreated EFB vermicompost, and untreated EFB vermicompost was measured by analysing thermogravimetric (TG) and derivative

thermogravimetric (DTG) curves. The thermal degradation temperature for holocellulose and lignin degradation in raw EFB obtained from TGA and DTGA curves were used as a control in TGA and DTGA analyses of pretreated EFB vermicompost and untreated EFB. The analyses were conducted using a Perkin Elmer Pyris 1 TGA (Shelton, USA) with nitrogen as the carrier gas at the temperature range of 50 °C to 850 °C and a heating rate of 10 °C min⁻¹. Nitrogen carrier gas was purged at a flow rate of 20 mL min⁻¹ from 50 °C to 700 °C. Next, the nitrogen was replaced with air at a similar flow rate (20 mL min⁻¹) from 700 °C to 850 °C. Data were analysed using Pyris software, and the weight loss related to the moisture content was evaluated as the weight that corresponded to a peak in the water mass spectra.

Scanning electron microscope analyses

The morphological changes of the vermicomposting of untreated and pretreated EFB were examined using a scanning electron microscope (SEM). A Jeol JSM- 6400 Scanning Microscope (Peabody, MA, USA) was used to study the effect of EFB vermicomposting in a LSVT for 4 replicates at an acceleration voltage of 15 kV. The raw EFB was ground to pieces of 5 mm to 10 mm using a Glen Creston Beater mill and was carefully dispersed on a carbon-coated tape that had been stuck to an aluminum stud with a diameter of 2.5 cm and a height of 1 cm. The surfaces of the samples were then sputter coated with gold (Sigma-Aldrich, St. Louis, MO, USA). The current was maintained at 15 mA and a pressure of ≥ 0.5 torr.

RESULTS AND DISCUSSION

Moisture contents in raw EFB, untreated EFB vermicompost, and pretreated EFB vermicompost were determined to be 70±1%, 54±2%, and 66±2%, respectively. Pattnaik and Reddy (2010) determined moisture content in urban green waste vermicompost to be 55 to 60%. The minimal moisture content requirement for microbial activity in vermicompost has been reported to be 50% (Liang *et al.* 2003). It was found that the moisture content in pretreated EFB vermicompost was higher than untreated EFB vermicompost, which might be due to the higher absorption capacity because of assimilation of higher microbial population during vermicomposting. However, the moisture contents of raw EFB, pretreated EFB vermicompost, and untreated EFB vermicompost were minimized *via* air dry prior to further analyses.

Thermogravimetric and Differential Thermal Analysis of EFB Vermicompost

Three distinct stages of weight loss of raw EFB are shown in Fig. 2. The first stage of weight loss at < 150 °C was due to the decrease in moisture content in the raw EFB (Fatah *et al.* 2014). In the DTG curve between 150 °C and 380 °C, there was a peak at 308 °C (Fig. 2). From the TG and DTG curves (Fig. 2), it is very difficult to distinguish between hemicellulose and cellulose. However, a 54.3% weight reduction of hemicellulose and cellulose was found in raw EFB at the decomposition temperature range of 150 °C to 380 °C. The weight reduction for lignin was measured as 12.4% between 380 °C and 700 °C. An 18.45% weight reduction for fixed carbons was observed between 700 °C and 800 °C. The results obtained in the present study were different from those obtained by Yang *et al.* (2007), who reported that the decomposition temperatures of hemicellulose and cellulose

were 220 °C to 315 °C and 315 °C to 390 °C, respectively. The differences in the decomposition temperatures of hemicellulose and cellulose might be due to differences in the age and type of the oil palm plantation (Omar *et al.* 2011).

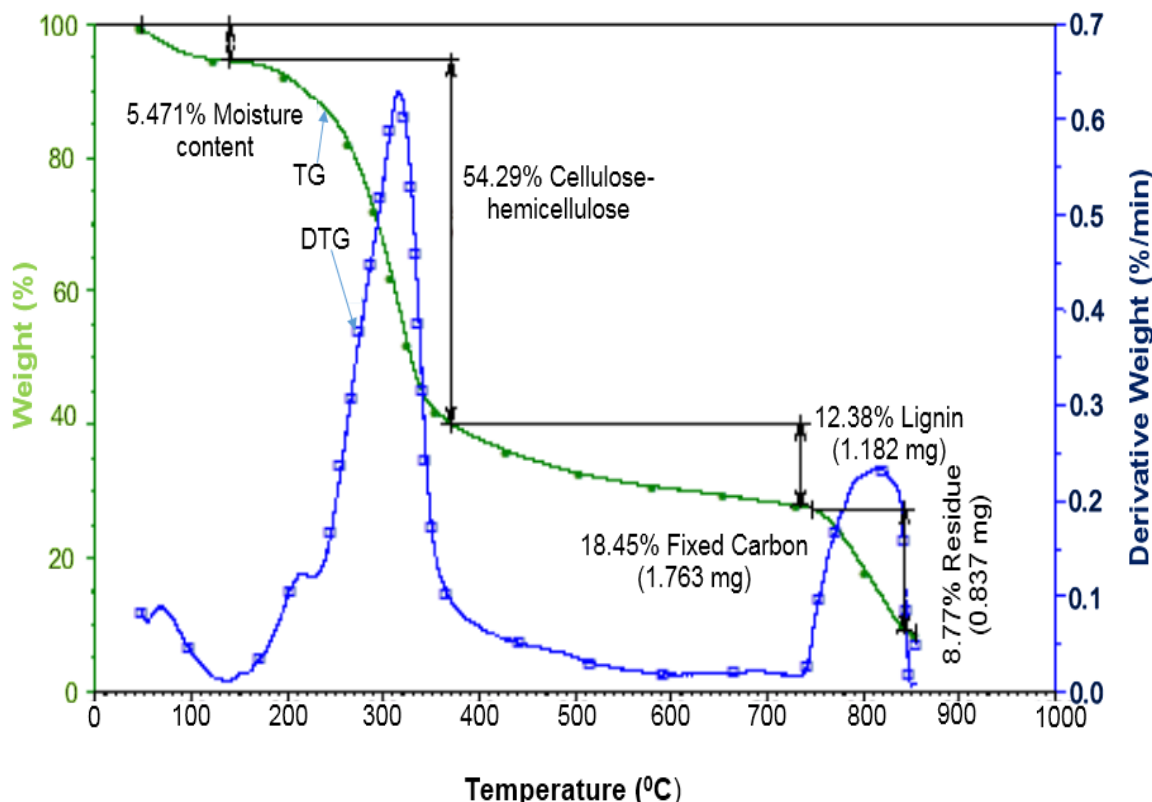


Fig. 2. Thermogravimetric and differential thermal analysis of raw EFB

The TG curve represents the instantaneous weight loss of the samples, as shown in Fig. 3. The degradation temperature for hemicellulose-cellulose from the LSVT was over 50 °C, which was higher than that of the raw EFB (Fig. 3). The degradation temperature increase for the EFB vermicompost was probably due to the deterioration of the lignocellulose complex during the 30 weeks of composting. The DTG curve in Fig. 4 represents the rate of weight loss for the LSVT samples. The weight loss of the EFB vermicompost began at 120 °C. The small peaks at 320 °C might have been the starting point of cellulose decomposition (Omar *et al.* 2011). The maximum weight loss occurred at 380 °C and the weight loss rate dropped to a low level ($< 0.5 \text{ wt.}\% \text{ min}^{-1}$) at over 400 °C, indicating that lignin was degrading very slowly. At 700 °C, the weight loss accelerated when oxygen was supplied at 10 mL min^{-1} to determine the amount of fixed carbon. Figure 3 shows that the carbonisation of fixed carbons stopped at approximately 800 °C. In contrast, the carbonisation of fixed carbons in raw EFB stopped at 850 °C (Fig. 4). The maximum peak at 785 °C for treated EFB indicated the maximum weight loss rate (between $2.75 \text{ wt.}\% \text{ min}^{-1}$ and $3.67 \text{ wt.}\% \text{ min}^{-1}$). The mass loss rate of the treated EFB was higher than that of raw EFB. The amount of fixed carbons in treated EFB was 16%, which was less than that of raw EFB in which the amount of fixed carbon was 18.45% (Fig. 3). The average ash content in all treated EFBs was 5%, which was less than the 8.8% ash content of raw EFB.

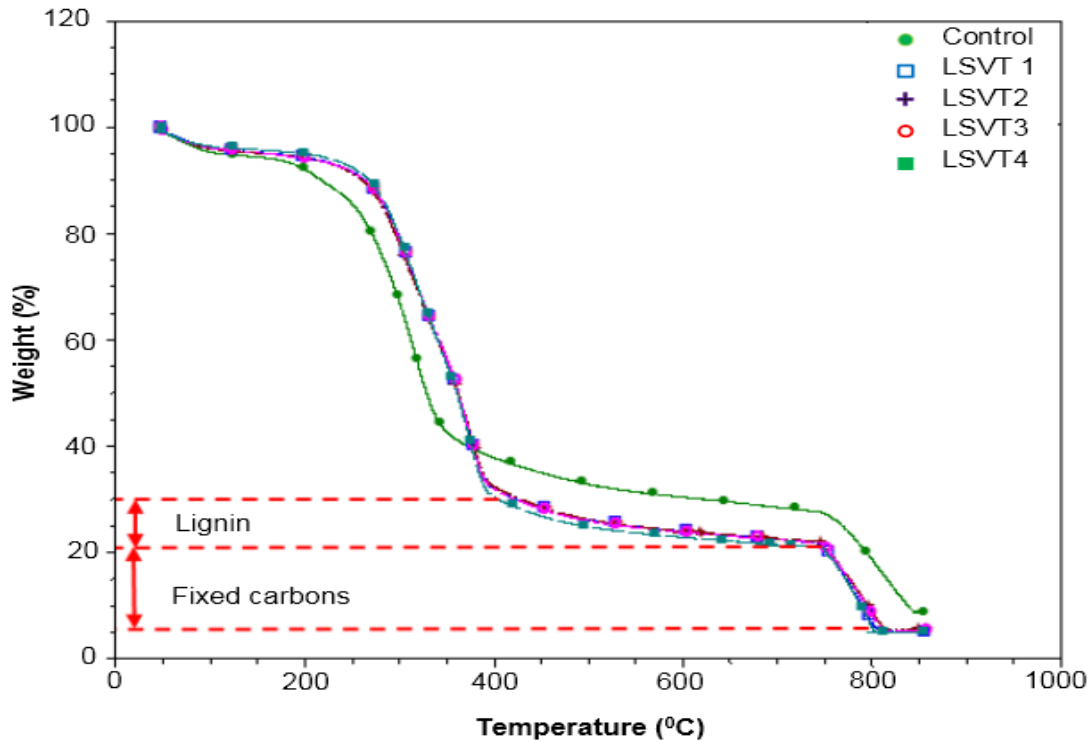


Fig. 3. Distributions of thermogravimetric analysis of EFB vermicomposting (untreated) in large-scale vermicomposting trials

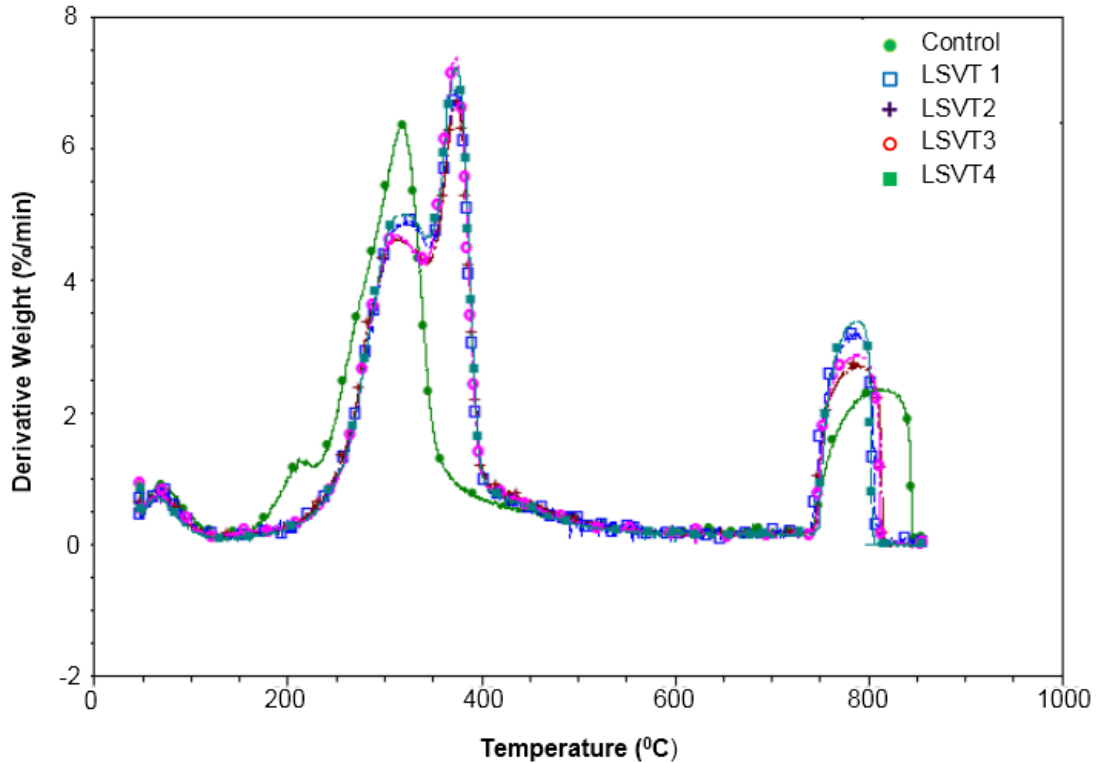


Fig. 4. Distribution of differential thermogravimetric analysis of EFB vermicomposting (untreated) in large-scale vermicomposting trials

Due to the degradation temperature shift in the LSVT trial samples it was difficult to determine the percentage of hemicellulose-cellulose left after cultivation. Omar *et al.* (2011) agreed that it was difficult to distinguish hemicellulose-cellulose through TGA analysis of treated lignocellulose waste and concluded that the thermal degradation of the biomass structure starts with hemicellulose, is followed by cellulose, and ends with lignin. Loss of lignin in the treated EFB from the LSVT trials can be seen by comparing the LSVTs of EFB vermicomposting to control data (Fig. 4). The loss of lignin in all untreated EFBs ended at 750 °C, which was earlier than raw EFB in which lignin decomposition ended at 780 °C. This indicated that the level of lignin in untreated EFB decreased during vermicomposting. The lignin content in EFB vermicomposting varied from 8.64% to 9.79%. In raw EFB vermicomposting the lignin content was 12.4%. The degradation of lignin in LSVT trials was lower than that of the control data as indicated by the difference between the dotted lines of weight loss shown in Fig 3. This was correlated with mass loss rate that was almost nil for the LSVTs samples compared to 2.3% min⁻¹ for the control.

The TGA and DTGA curves of treated EFB vermicompost at each LSVT are shown in Figs. 5 and 6, respectively. The maximum weight loss for treated EFB vermicompost was at 335 °C with a weight loss rate between 0.15% min⁻¹ and 0.27% min⁻¹. The weight loss rate remained at 0.05% min⁻¹ until carbonisation began at 700 °C. The ash contents of LSVT 1 (Vermi 1), LSVT 3 (Vermi 3), and LSVT 4 (Vermi 4) were higher than that of raw EFB (Fig. 5).

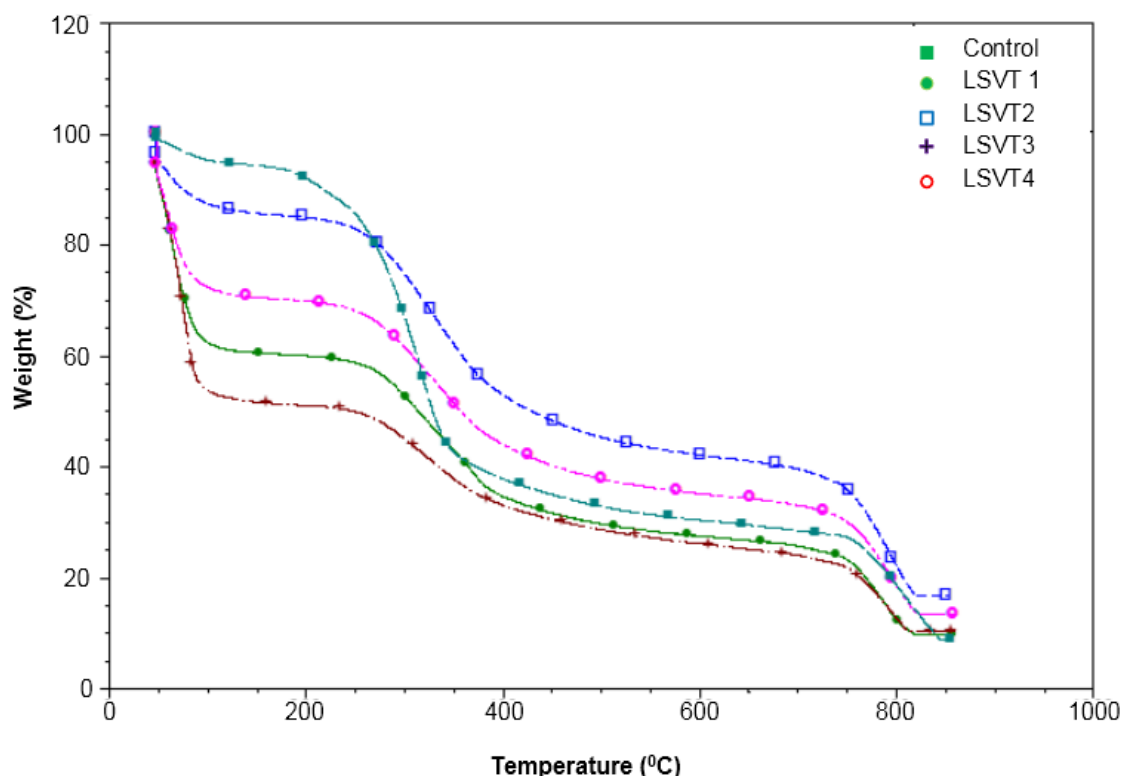


Fig. 5. Distributions of thermogravimetric analysis of treated EFB vermicomposting in large-scale vermicomposting trials

It was difficult to measure the amount of hemicellulose, cellulose, and lignin left after vermicomposting *via* TGA analysis. The progressive reduction of the weight of the

EFB vermicompost was associated with a peak from 220 °C to 390 °C, which indicated the progressive degradation of aliphatic compounds, carbohydrates, and some easily degradable aromatic compounds (lignin). Moreover, the thermal degradation of treated EFB vermicompost was higher than that of the untreated EFB vermicompost and raw EFB. The reduction of lignin in the treated EFB varied from 21% to 30% for LSVT 3 and LSVT 1, respectively.

The composition of hemicellulose, cellulose, and lignin in EFB is comparable to other substrates used for *P. sajor-caju* cultivation (Stamets and Chilton 1983; Kume *et al.* 1998). According to Stamets and Chilton (1983), the decomposition of lignin to a nitrogen-rich lignin humus complex is a promising way to produce protein. Therefore, EFB is a suitable substrate with ideal structural and chemical properties for mushroom cultivation. Kumar *et al.* (2010) concluded that pre-composting and co-composting material high in lignocellulose improves the survival rate of earthworms (*E. fetida*) and accelerates vermicomposting. This is an indicator that earthworm activity has broken down the hemicellulose, cellulose, and lignin in EFB during the LSVTs in treated EFB vermicomposting. This is consistent with the findings of Rani *et al.* (2008) who found that 23% to 30% of lignin in paddy straw and sorghum stalk degraded during the cultivation of *P. sajor-caju*.

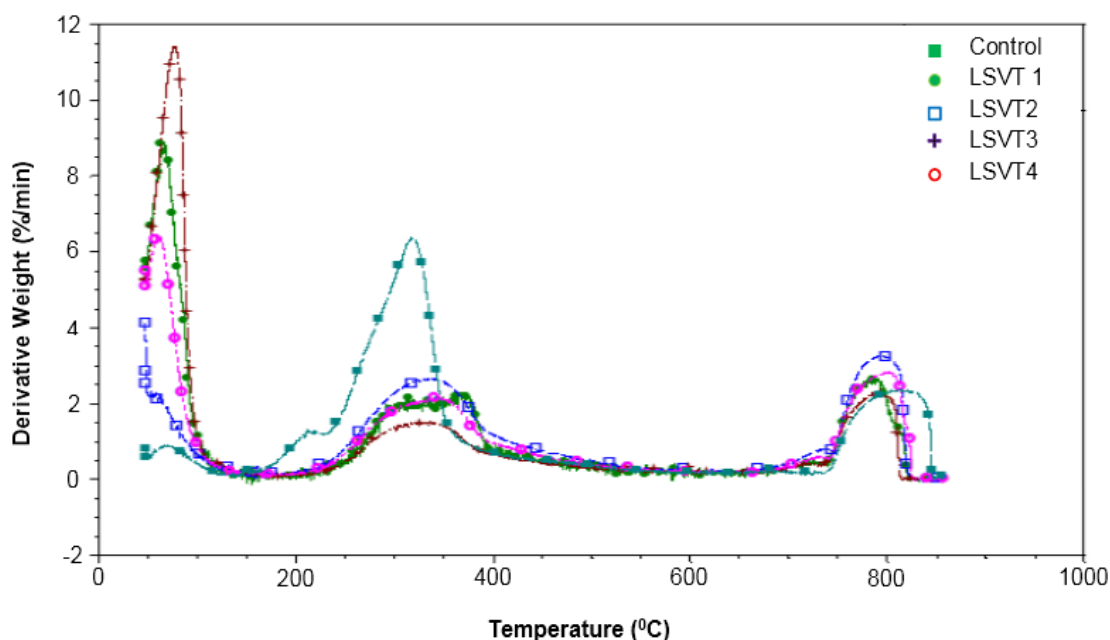


Fig. 6. Distributions of DTGS of treated EFB vermicomposting in large-scale vermicomposting trials

Morphological Changes of Treated EFB throughout Vermicomposting

Figure 7 shows the physical changes of EFB from raw material to 30-week vermicompost. It was observed that the colour of raw EFB (Fig. 7a) changed from bright orange-brown to pale-greyish-brown after the cultivation of *P. sajor-caju* (Fig. 7b). The treated EFB structure was more varied and less consistent (softer with thinner fibres) than the raw EFB fibre. This was due to the decomposition of cellulose, hemicellulose, and lignin, which make it suitable for vermicomposting. Kumar *et al.* (2010) found similar results, where *P. sajor-caju* was grown with other fungi to accelerate the degradation of cellulose, hemicellulose, and lignin from sugar-cane waste and wheat straw before

vermicomposting. Figure 8c shows the vermicasts with almost cylindrical shapes and a dark brown colour. The length of each vermicast was approximately 3 mm. It can also be seen that there was a mix of cast and fibre attached together. It seems that not all of the fibre was digested by earthworms after passing through their bodies. However, as no additional fresh feed was supplied to the earthworms, the earthworms used the initial vermicast as a food source produced a more refined vermicast. After 30 weeks of vermicomposting, the shape of the vermicast became finer and the colour darkened (Fig. 8d). Further degradation of fibre was also due to the attachment of micro-flora from the earthworms (Hayawin *et al.* 2011; Sabrina *et al.* 2011).



Fig. 7. Physical changes of EFB from raw material to vermicompost; (a) raw EFB (control), (b) treated EFB with *P. sajor-caju*, (c) vermicasts, and (d) EFB vermicompost after 30 weeks of composting

SEM Analyses of EFB Vermicompost

Scanning electron microscopy was used to observe the effect of earthworms on the treated and untreated EFB vermicomposting, as presented in Fig. 8d. The SEM image of untreated EFB vermicompost showed that the treated EFB vermicomposted fibres were more scattered than those of raw EFB. However, the surface of the pretreated EFB vermicompost became rough and fragmented with holes, which created a larger surface area and may have promoted surface adhesion for microbes in the earthworm gut and micro-flora, causing further degradation (Fig. 8c). This may have occurred due to the

complete degradation of cellulose and hemicellulose in the treated EFB vermicompost. Similar observations were reported by Mohamad Haafiz *et al.* (2015), who studied the removal of cellulose and hemicellulose from treated EFB when creating an EFB-polypropylene composite and Polymeric-EFB. Figure 8d shows a honeycomb-like structure on the surface of the SEM image of a cross-section of treated EFB vermicompost. This may have been attributed to the fact that xylem structures are ruptured due to the release of enzymes by *P. sajor-caju*. In addition, microbial and microflora from the earthworm gut may attack xylem structures. Moreover, the xylem tubes became rougher due to microflora activity and digestion and degradation by enzymes. The rupture of the xylem structure is an indicator of lignin decomposition. This is because xylem is made of lignin, which provides strength and protection from microbial attack (Voelker *et al.* 2011).

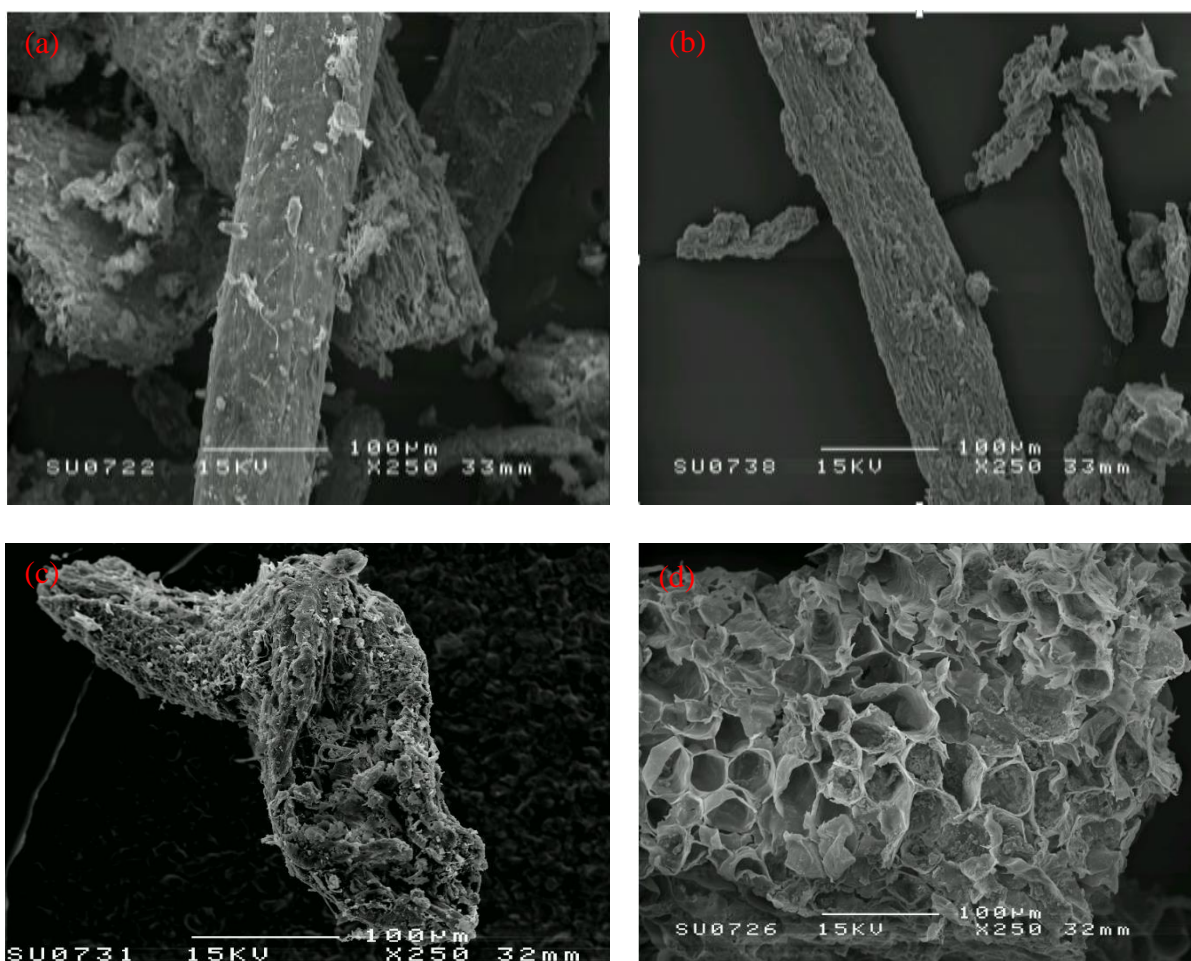


Fig. 8. Morphology changes of raw EFB and EFB vermicompost. (a) raw EFB, (b) untreated EFB vermicompost, (c) pretreated EFB vermicompost, and (d) cross-section of pretreated EFB vermicompost

CONCLUSIONS

1. Moisture contents in untreated EFB vermicompost and pretreated EFB vermicompost were determined to be $70\pm 1\%$, $54\pm 2\%$ and $66\pm 2\%$, respectively.

2. The weight reduction of hemicellulose and cellulose in raw EFB was 54.3% at a decomposition temperature between 150 °C and 380 °C. The weight reduction for lignin in raw EFB was measured as 12.4% between 380 °C and 700 °C.
3. The amount of lignin in EFB was reduced from 12.4% to 8.64% during vermicomposting.
4. The progressive weight reduction of the EFB vermicompost was associated with a peak from 220 °C to 390 °C. The thermal degradation of treated EFB vermicompost was higher than that of the untreated EFB vermicompost and raw EFB. The reduction of lignin in the treated EFB varied from 21% to 30% for LSVT 3 and LSVT 1, respectively.
5. The colour of raw EFB changed from bright orange-brown to pale-greyish-brown after being treated with *P. sajor-caju*. However, the shape of the treated EFB vermicast became finer and darkened in colour after 30 weeks of vermicomposting.
6. The SEM image of EFB vermicompost showed distinct changes in physical appearance. The surface of the SEM image for EFB vermicompost was scattered. The surface of the treated EFB vermicompost became rough and fragmented with holes present.

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