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ORIGINAL ARTICLE

Solid-state fermentation of oil palm frond petiole for lignin peroxidase and xylanase-rich cocktail production

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Abstract

In current practice, oil palm frond leaflets and stems are re-used for soil nutrient recycling, while the petioles are typically burned. Frond petioles have high commercialization value, attributed to high lignocellulose fiber content and abundant of juice containing free reducing sugars. Pressed petiole fiber is the subject of interest in this study for the production of lignocellulolytic enzyme. The initial characterization showed the combination of 0.125 mm frond particle size and 60% moisture content provided a surface area of 42.3 m^2/g, porosity of 12.8%, and density of 1.2 g/cm^3, which facilitated fungal solid-state fermentation. Among the several species of *Aspergillus* and *Trichoderma* tested, *Aspergillus awamori* MMS4 yielded the highest xylanase (109 IU/g) and cellulase (12 IU/g), while *Trichoderma virens* UKM1 yielded the highest lignin peroxidase (222 IU/g). Crude enzyme cocktail also contained various sugar residues, mainly glucose and xylose (0.1–0.4 g/L), from the hydrolysis of cellulose and hemicellulose. FT-IR analysis of the fermented petioles observed reduction in cellulose crystallinity ($I_{900/1098}$), cellulose–lignin ($I_{900/1511}$), and lignin–hemicellulose ($I_{1511/1738}$) linkages. The study demonstrated successful bioconversion of chemically untreated frond petioles into lignin peroxidase and xylanase-rich enzyme cocktail under SSF condition.

Keywords Oil palm fronds · Solid-state fermentation · *Aspergillus* sp. · *Trichoderma* sp. · Lignocellulolytic enzymes

Introduction

Malaysia is the second largest producer of palm oil after Indonesia, with production capacity of 17.32 million tonnes over a cultivated area of 5.74 million ha (Rozali et al. 2017). Palm trees are mainly cultivated for their fruits, which merely account for 10% of entire tree, while the remaining 90% are agro-residues in the form of oil palm fronds (OPF), empty fruit bunches (EFB), and oil palm trunks (OPT) (Sukiran et al. 2017). The Malaysian oil palm residues are projected to hit a 100 million tonnes by 2020. Recognizing the growing waste, the government initiated the National Biomass Strategy, intending to convert abundant biomass wastes into value-added products, including biofuel, biofertilizer, enzymes, and other bio-based chemicals.

Oil palm wastes consist of rich cellulose, hemicellulose, and lignin content, which are primary sources for cellulase, xylanase, and ligninolytic enzyme production. Studies indicated that enzyme production is the fastest growing field in biotechnology with annual sales reaching USD 4.2 billion (Singh et al. 2016). OPF is the most abundant residue of palm trees, amounting up to 70% of the total palm waste. Attributed to this, OPF is available at a cheap cost of USD 20 for every tonne. Compared to other oil palm waste, the present application of OPF is limited to animal feed and soil replenishment (Ishida and Abu Hassan 1997; Islam et al. 2000), while excess components are disposed through burning (Lee et al. 2016). These factors appeal researchers to explore OPF biomass for enzymatic study. Despite this, several studies reported OPF application as unfavourable in fermentation due to high silica and lignin content (Dahlan 2000; Yunus et al. 2010). However, these studies are only limited to compositional assessments, which lack in fermentation findings related to lignocellulolytic production.

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Lignocellulolytic mixture is a multi-enzyme extract consisting of seven major enzymes, including: endoglucanase (EC 3.2.14), endoxylanase (EC 3.2.1.8), β-glucosidase (EC 3.2.1.21), exoglucanase (EC 3.2.1.91), lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), and laccase (EC 1.10.3.2). Such diverse enzyme mixture is produced when fermentation is carried out on a heterogenous substrate (i.e., oil palm biomass) and is usually present in varying compositions, depending on the capability of the fermenting microorganism used. Studying enzyme profiles would help to assess the potential of the individual enzyme and its interaction in the mixture, as well as, its application in various fields (Mansour et al. 2016).

Filamentous fungi such as *Aspergillus* and *Trichoderma* species have been widely studied under solid-state fermentation (SSF) for various enzyme productions. However, most studies investigated the production of hydrolytic enzymes such as cellulase and xylanase, with a little attention given to ligninolytic enzymes. In light of this, advances made in examining all enzymes in a lignocellulolytic mixture would be highly beneficial especially, since, SSF is a proven system; capable of producing high titres of enzymes at lower cost (Ang et al. 2015; Pandey 2003). In spite of the vast opportunity, successful SSF enzyme production requires careful evaluation of the physicochemical factors, such as carbon source, particle size, moisture content, temperature, and pH levels. This study reports on the characterization of OPF for enzyme production using six filamentous fungi from different species of *Aspergillus* and *Trichoderma*. Observations were made on the influence of pressed OPF petioles on fungal growth and enzyme production. To the extent of authors' knowledge, this is the first report that examines a complete lignocellulolytic profile from OPF fermentation.

Materials and methods

Microorganism and inoculum preparation

Six locally isolated fungal strains, *Trichoderma asperellum* MR1 (KP883284), *Trichoderma viride* MMS3 (KF691813), *Trichoderma virens* UKM1 (KR259658), *Aspergillus niger* EFB1 (KF691808), *Aspergillus fumigatus* SK1 (JQ665711.1), and *Aspergillus awamori* MMS4 (KF691812), were obtained from Biorefinery Technology Laboratory (BTL) culture collections, and used in this study. In inoculum preparation, the strains were cultured on potato dextrose agar (PDA) for 7 days at room temperature, which were harvested using Tween-80 1% (*w*/*v*), and centrifuged at 4000 rpm for 20 min. Spore pellets were mixed with sterile distilled water. Subsequently, spore count was performed using a haemocytometer to achieve the desired spore concentration of 10^8 spores/g of OPF. The preparation of the inoculum had adopted the methods reported in Ang et al. (2013).

Substrate preparation, size separation, and physical pre-treatment

Oil palm fronds (OPF) used in this study were obtained from a private oil palm plantation in Kota Tinggi, Johor, Malaysia. Leaflets and stems were removed from the OPF and only the petiole section was used in the study (Fig. 1). The OPF petiole was pressed and de-juiced using a sugarcane juicing machine (Hisaki, TFS3777). The petiole was repeatedly pressed and de-juiced, for three times, to completely remove the juice content. Next, the pressed OPF petiole was retrieved and cleaned with tap water. Size reduction of the petiole was performed using industrial grade grinder supplied by Biotrade Sdn Bhd. Subsequently, electric sieve with shaker (Endecotts, Minor) was used to separate ground OPF petiole fibers (0.125–0.500 mm). Prior to fermentation, OPF was autoclaved with temperature 121 °C at 15 psi for 15 min.

Solid-state fermentation and enzyme extraction

Solid-state fermentation (SSF) was carried out in a 100 × 15 mm soda-lime glass Steriplan™ petri dish (Duran). Prior to SSF, 15 g of sterile OPF was uniformly mixed with modified Mandel's medium and fungal inoculum (10^8 spores/g), to achieve a 60% moisture content. Modified Mandel's medium was prepared using the following chemicals: 1.4 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, 0.3 g/L urea, 0.3 g/L $CaCl_2$, 0.3 g/L, $MgSO_4$, 0.005 g/L $FeSO_4$, 0.0016 g/L $MnSO_4{\cdot}H_2O$, 0.0014 g/L $ZnSO_4{\cdot}7H_2O$, 0.002 g/L $CoCl_2$, 0.75 g/L peptone, and 2 mL/L Tween-80 (Mandels and Weber 1969). SSF was carried out under non-optimized condition at 35 °C for 7 days. Enzyme extraction was performed by mixing 4 g of fermented OPF in 100 ml of sodium acetate buffer (0.05 M). Later, the mixture was vortexed for 2 min. The mixture was then centrifuged at 4000 rpm with temperature 4 °C for 20 min. The supernatant containing crude enzyme mixture was filtered, analyzed for enzyme activities, and retained at − 20 °C for further non-enzymatic analysis.

Analytical procedures

Determination of average particle size

150 g of OPF sample was placed on the upper deck of sieve (0.500 mm) and was sifted for 10 min. Each sieve (hole diameter = 0.125, 0.250, and 0.500 mm), holding respective OPF particle sizes, was then weighed. Average particle size (d_{gw}) and its standard deviation (S_{gw}) were determined using the following equations, as described by Pfost and Headley (1976), where W_i is the weight of retained solid particles in every

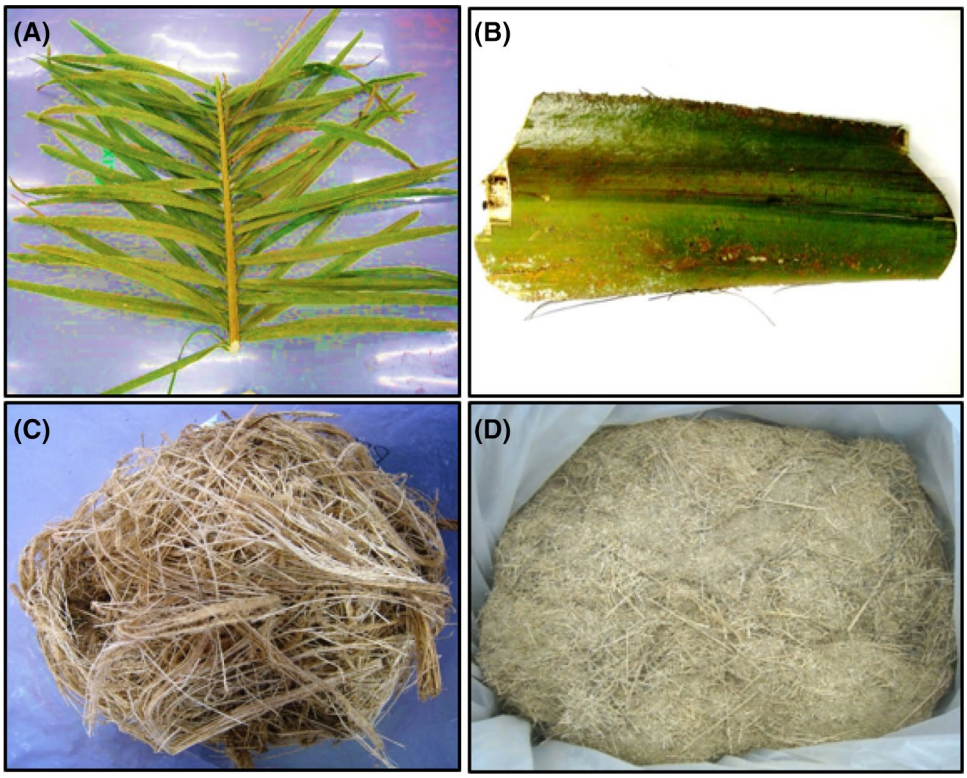


Fig. 1 Images of oil palm frond: **a** Upper section (leaflets and stem) high in soil nutrient; **b** basal section (petiole) high in fiber and juice with sugar content up to 171 mg/g; **c** pressed, washed, and dried petiole with reduced sugar content; **d** ground petiole fiber with reduced size (0.106–0.800 mm)

sieve, in unit gram; and d_i is the diameter of the sieve in millimeter (mm):

$$d_{\mathrm{gw}} = \log^{-1}\left(\frac{\sum(W_i \, x \log d_i)}{\sum W_i}\right) \quad (1)$$

$$S_{\mathrm{gw}} = \log^{-1}\left(\frac{\sum W_i \, (\log d_i \, x \log d_{\mathrm{gw}})2}{\sum W_i}\right)^{0.5}. \quad (2)$$

Determination of moisture content

Moisture content was determined using a moisture analyzer (A&D Company limited, MX-50). 10 g of moist substrate was placed onto the sample's pan. Next, heating was performed at 105 °C. The final moisture content was automatically computed in percentage.

Determination of density and porosity

Porosity was calculated based on the density of substrate. Bulk density was determined using the method described in Abalone et al. (2004), where a certain amount of solid substrate was mixed into a measuring cylinder of known volume and subsequently weighed. Bulk density (ρb) was determined using the following equation:

$$\text{Bulk density } (\rho b) = \frac{\text{mass of dry solid substrate (g)}}{\text{total volume of solid substrate and air (mL)}}. \quad (3)$$

Particle density (ρp) was determined based on soil particle density protocol, as described in Globe (2005). 12 g of solid substrate was mixed with 50 mL of distilled water in a 10 mL volumetric flask. The mixture was boiled for 10 min. The flask was then allowed to cool at room temperature for 24 h. Additional distilled water was added to achieve a 100 mL volume. Subsequently, the mixture was weighed. Particle density (ρp) and porosity were then determined using the following equations:

$$\text{Particle density } (\rho p) = \frac{\text{mass of dry solid substrate (g)}}{\text{Volume of solid substrate particles (mL)}} \quad (4)$$

$$\text{Porosity} = 1 - \left(\frac{\rho p}{\rho b}\right) \times 100. \quad (5)$$

Determination of surface area

Surface area was calculated using the following equation, as described in Pfost and Headley (1976), where βs is the shape factor for calculating surface area of particles; βv is the shape factor for calculating volume of particles; ρp is the particle density of solid substrate (g/cm^3); d_{gw} and S_{gw} is the average particle size and its standard deviation:

$$\text{Surface area} = \left(\frac{\beta s}{\rho p \beta v}\right) \exp\left(0.5 \, \mathrm{In}^2 S_{\mathrm{gw}} - \mathrm{In} \, d_{\mathrm{gw}}\right). \quad (6)$$

Biomass estimation

Fungal biomass was estimated using modified N-acetylglucosamine method, as described in Sakurai et al. (1977). The method involves the quantification of glucosamine that is released from chitin. 0.1 g of fermented OPF was mixed with 5 mL of hydrochloric acid (2 M) and boiled for 2 h. Two drops of phenolphthalein and several drops of sodium hydroxide (NaOH) 1 M were added to turn the mixture into pink color. Neutralization was then performed by adding several drops of KH_2PO_4 1.0% (*w*/*v*) until the mixture was decolorized. Next, the mixture was boiled for 20 min, followed by, adding 6 mL of absolute ethanol and 1 ml of Ehrlich reagent. The mixture was later heated at 65 °C for 15 min and measured for glucosamine.

Total reducing sugar

Dinitrosalicylic acid (DNS) method was used for the detection of reducing sugars based on the procedures described by Miller (1959). Following the procedures, 1 mL of sample, 1 mL of DNS, and two drops of sodium hydroxide (NaOH) 0.1 M were mixed and boiled for 5 min. The boiled sample was allowed to cool to room temperature and measured for reducing sugars.

Cellulolytic and hemicellulolytic assays

Endoglucanase and xylanase activities were measured based on the hydrolysis of 0.5 ml enzyme, with 0.5 mL 2% (*w*/*v*) carboxymethyl cellulose (CMC) and 0.5 mL 1% (*w*/*v*) xylan, respectively. The incubation time was set to 30 min. For exoglucanase, 0.5 mL enzyme was reacted with 50 mg Whatman filter paper (10 × 60 mm), in 1 mL sodium acetate buffer (0.05 M, pH 5) for 60 min. Upon completion of the reaction, all three assays were boiled with 1 mL of dinitrosalicyclic acid (DNS) and two drops of sodium hydroxide (0.1 M), and subsequently, the release of reducing sugars was measured (Ghose 1987; Ghose and Bisaria 1987). β-glucosidase activity was determined based on the reaction of 0.5 mL enzyme with 1 mL *p*-nitrophenyl-β-D-glucopyranoside (pNPG) for 30 min. The reaction was stopped using 2 mL glycine buffer (0.4 M, pH 10.8), and subsequently, the release of *p*-nitrophenol was measured. One unit of endoglucanase and exoglucanase corresponds to 1 µmole of glucose released per minute. One unit of xylanase activity was defined as the releasing of 1 µmole of xylose per minute.

Ligninolytic assay

Three ligninolytic assays consisting of lignin peroxidase (Tien 1988), manganese peroxidase, (Yang et al. 2011), and laccase (Karp et al. 2012) were analyzed. For lignin peroxidase, 0.5 mL of enzyme was mixed with, 0.4 mL of sodium acetate buffer (0.05 M, pH 5), 0.1 mL of veratryl alcohol (20 mM), and 40 µL of hydrogen peroxide (20 mM), prior to incubation at 37 °C for 3 min. For manganese peroxidase, 0.5 mL of enzyme was mixed with, 0.1 mL phenol red, 0.1 mL of manganese(II) sulfate monohydrate (5 mM), 1.2 mL sodium acetate buffer (0.05 M, pH 5), and 0.1 mL of hydrogen peroxide (2 mM), before incubation at 30 °C for 60 min. Meanwhile, laccase activity was determined by mixing 0.1 mL of enzyme with 0.2 mL 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS (20 mM), and 1.7 mL of sodium acetate buffer (0.05 M, pH 5), before incubation at 30 °C for 60 min. Consequently, the optical density of lignin peroxidase, manganese peroxidase, and laccase was measured at 310, 610, and 420 nm, respectively.

High-performance liquid chromatography (HPLC)

HPLC analysis was performed to detect polyoses, including arabinose, maltose, fructose, xylose, galactose, cellobiose, and glucose. The analysis had utilized Agilent HPLC 1100, equipped with Phenomenex's Rezex RPM-Monosaccharide Pb^{+2} column with refractive index detector (RID). Furthermore, mobile phase had utilized 100% ultrapure water with a flow rate of 0.3 mL/min and temperature 80 °C. Determination of the polyoses content was adopted from Noratiqah et al. (2013).

Fourier transform infrared spectroscopy (FT-IR)

An FT-IR spectrophotometer (Thermo Scientific, Nicolet iS5) with iD5-attenuated total reflectance (ATR) accessory was used for the detection of functional groups. Sample spectra were measured in terms of percentage transmittance in a frequency range of 800–4000/cm with spectral resolution of 4.0/cm.

Scanning electron microscopy (SEM)

Scanning electron microscope (Hitachi, SU1510) was utilized to examine samples. Samples were coated with gold and subsequently observed at a magnification range of 500–6000x.

Statistical analysis

Experimental result is presented in means ± standard deviation (SD) of two or three replicates. One-way analysis of variance (ANOVA) was performed and significant differences ($p < 0.05$) were determined following Duncan multiple range post hoc tests using SPSS version 17.

Result and discussion

Substrate characterization

OPF possesses rich cellulose and hemicellulose composition representing 65% of the total fiber content (Table 1). In SSF enzyme production, the lignocellulose composition is an important consideration, as it is the main carbon source and site for fungal attachment. In the study, only OPF petiole was used as a substrate (Fig. 1), as the leaflets and stems are rich in nutrients, such as nitrogen, phosphorus, potassium, and magnesium. These nutrients are better suited to be used in recycling soil nutrients and fortifying conservation efforts (Tan et al. 2017). The removal reduced lignin composition by 9.5%, making the petiole's lignin content as the lowest when compared against other oil palm residues (Table 1). Lignin is non-hydrolysable and its high presence limits enzymatic hydrolysis of cellulose and hemicellulose.

In addition, OPF also possesses a high ash content (silicon, chlorine, and sulphur), compared to EFB and OPT (Sorek et al. 2014; Roslan et al. 2014). Among which, silica (or silicone dioxide, SiO_2) is highly present and its accumulation forms silica bodies on the fiber surface, as shown in Fig. 2a (Chen 2014). Despite playing a vital role in structural function of plants, in fermentation, silica adds rigidity to fiber, which consequently limits enzyme penetration and fiber degradation (Dahlan 2000). Earlier, simple physical pre-treatments, such as pressing, sizing, and autoclaving, were performed to petioles. The pre-treatments were intended to increase susceptibility of cellulose and hemicellulose in petioles, in addition to reducing the influence of silica and other inhibiting factors such as high-sugar concentration (Zahari et al. 2012; Yunus et al. 2010). Pressing was performed to remove OPF juice due to its high concentration of free reducing sugar at 171 mg/g. High-sugar concentration resulted in poor fungal growth and enzyme production as observed in the initial study. Such condition inhibits fungal growth due to hypertonic environment that disrupts cell function (Díaz et al. 2012). In addition, the petiole fibers are washed with tap water to ensure complete removal of residual sugars. As a result, this had effectively reduced 90% of the reducing sugar concentration to merely 13.8 mg/g. Grinding was performed to reduce the size of pressed OPF petiole into small particles of 0.125–0.500 mm. Consequently, the grinding had effectively exposed a larger collective surface

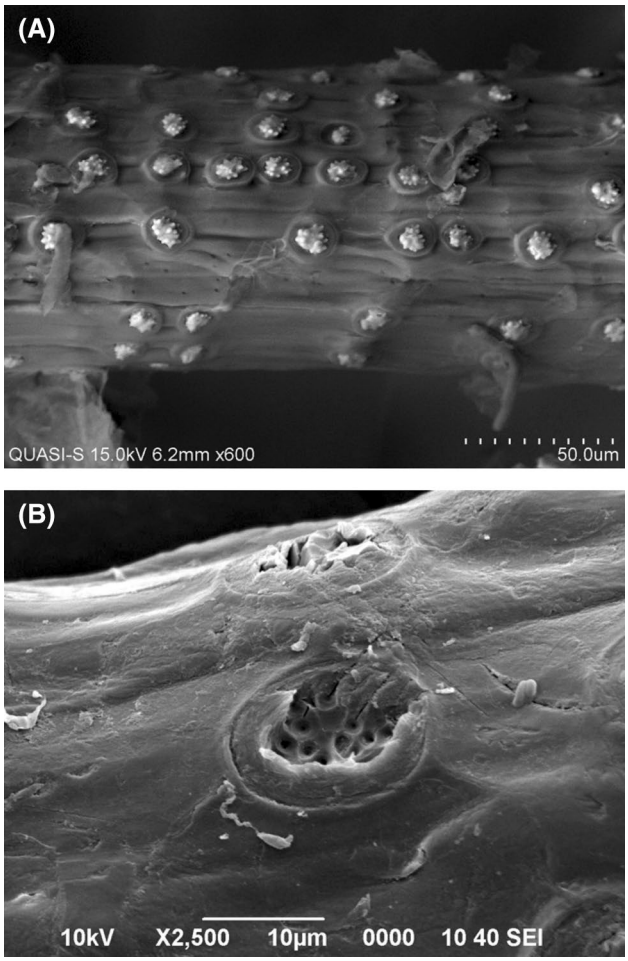


Fig. 2 Scanning electron microscope (SEM) images of raw OPF petiole with silica bodies (**a**) and autoclaved pressed petiole fiber with detached silica (**b**)

Table 1 Chemical composition of oil palm biomass residue

Oil palm biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
EFB[a]	50.5	29.6	17.8	3.4
OPT[a]	41.0	32.0	24.5	2.2
OPF[a]	56.0	28.0	20.5	2.4
Raw OPF (petiole)[b]	42.1	23.1	10.5	5.5
Pre-treated OPF (petiole)[b]	44.1	27.3	10.1	4.9

[a]Abdul Khalil et al. (2008)
bThis work

area of ground OPF petiole fibers for microbial attack. Comparable studies using oil palm wastes also ground waste into small particle sizes with similar ranges (Ang et al. 2013; Musa et al. 2017). Meanwhile, autoclaving was primarily performed for sterilization purposes, which inevitably served as a form of hydrothermal pre-treatment, as well. The process did not only fractionate lignocellulose by loosening hemicellulose and lignin linkages, but also contributed to the detachment of silica (Fig. 2b), which could further improve absorption and penetration of enzyme (Yunus et al. 2010). Indeed, the pre-treatments had increased cellulose and hemicellulose content, while at the same time had reduced lignin and ash content.

Effect of particle size

Three particle sizes used in this study: 0.125, 0.250, and 0.500 mm have a surface area of 36, 17, and 11 m^2/g, respectively. The smallest particle size (0.125 mm) was selected as it achieved a high fungal growth of 87.3 mg/g compared to 61.2 mg/g (0.250 mm) and 47.8 mg/g (0.500 mm) observed during the initial fermentation. Small particle sizes with large surface area provide a higher prospect for fungal attachment, and with good diffusion of air, water, and metabolites, causes a better fiber degradation (Manpreet et al. 2005).

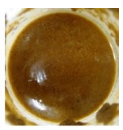
Effect of different moisture content

Table 2 shows the effect of OPF petiole at different moisture contents. SSF requires the selection of suitable moisture for proper fungal metabolism (Manpreet et al. 2005). Based on qualitative observation, the OPF samples can be separated into four moisture categories; low (7–45%), moderate (55–60%), high (70–75%), and extreme (80%). At lower moisture, the OPF appears dry and risks drying prematurely from evaporation during fermentation. It also has low surface area with high particle density (Table 2). At high moisture (70–75%), OPF particles appear agglomerated. This reduces interparticle spaces, density, and porosity value of the fiber. At 80% moisture, the OPF particles are completely filled with water in free flowing manner, which further reduces particle density. This significantly impacted the porosity and the overall physical appearance, which do not fit the characteristic definition of a solid substrate (Bhargav et al. 2008). Moderate moisture contents at 55 and 60% were strongly considered based on their physical appearance, which possessed sufficient moist, without agglomeration, and had more balanced surface area and porosity (Table 2). In determining the best moisture content, a preliminary fermentation was performed. The result indicated that OPF with 60% moisture observed lower but more regular water loss compared to OPF with 55% moisture. Moisture loss during fermentation is inevitable due to evaporation and accumulation of unventilated heat from metabolic activity (Bhargav et al. 2008). In SSF, maintaining a stable moisture throughout the fermentation period is crucial to avoid any reduction in enzyme activity (Amorim et al. 2017).

Fungal growth

Figure 3 shows the fungal growth of all six *Aspergillus* and *Trichoderma* species with maximum biomass production in the range of 76–101 mg/g. *A. awamori* MMS4 and *A. fumigatus* SK1 achieved the highest average biomass production of 59 and 55 mg/g, respectively, with a short doubling time of 0.056/h. Among the *Trichoderma* species, *T.viride* MMS3 was the best performing fungus at 51 mg/g, followed by *T. virens* UKM1 50 mg/g, and *T. asperellum* MR1 48 mg/g. These fungi recorded moderate growth rates between 0.061 and 0.066/h. Meanwhile, *A.niger* EFB1 recorded the lowest biomass production of

Table 2 Effect of different moisture contents on physical properties of pressed OPF petiole (0.125 mm)

								
Moisture content (%)	7%	30%	45%	55%	60%	70%	75%	80%
Surface area (m^2/g)	36.3 ± 0.78	38.6 ± 0.50	40.5 ± 0.78	41.9 ± 0.35	42.3 ± 0.81	44.2 ± 0.77	44.6 ± 0.13	46.7 ± 0.84
Porosity (%)	25.3 ± 0.67	20.8 ± 0.99	16.9 ± 0.99	13.8 ± 1.24	12.8 ± 0.92	8.5 ± 0.78	8.0 ± 1.06	0.00 ± 0.35
Particle density (g/cm^3)	1.35 ± 0.50	1.27 ± 0.14	1.21 ± 0.14	1.17 ± 0.14	1.16 ± 0.28	1.11 ± 0.50	1.10 ± 0.78	1.05 ± 0.35

All values are average ± standard deviation of mean from duplicate sample

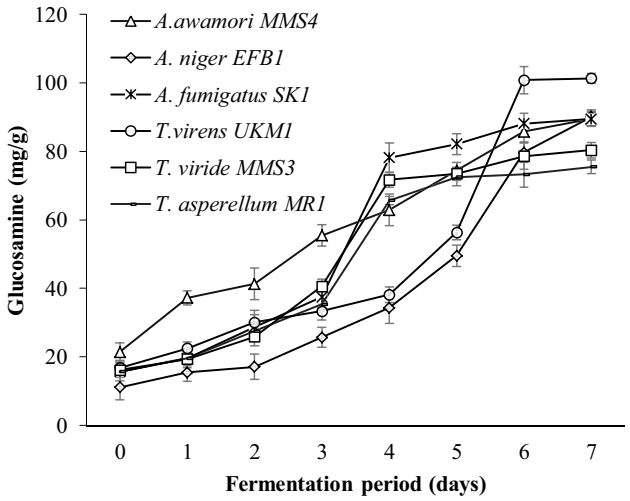


Fig. 3 Fungal growth on pressed OPF petiole. Error bars indicate standard deviation of mean for sample in duplicates

40 mg/g and the slowest growth rate of 0.073/h. Fungal growth rates and biomass production during SSF are heavily influenced by physicochemical factors such as variations in temperature and pH levels. However, both parameters were not optimized in this study. Still, the temperature is within the reported optimal temperature for *Aspergillus* species (30–37 °C), while slightly higher for *Trichoderma* species (25–30 °C) (Krijgsheld et al. 2013; Singh et al. 2014). The initial pH 5 was based on the pH of Mandel's medium. However, during fermentation, reduction in pH (pH 2.5–3) was observed between 0 and 3 days, which later gradually stabilized to pH 4.5-5 from day 4 onwards. The drop in pH is most likely attributed to the formation of organic acids (i.e. acetic acid) and the consumption of ammonium salt in the medium (Yoon et al. 2014). As for pH, both *Aspergillus* and *Trichoderma* species have a wide range of reported optimum pH levels ranging from pH 2–6 (Kredics et al. 2003).

Overall, the positive biomass growth observed in all strains indicates that selected fungi subjected under the following operating conditions; particle size (0.125 mm), moisture content (60%), temperature (35 °C), and pH (5.0), supported fungal growth with OPF petiole acting as the sole carbon source. The biomass produced in this study was comparable with the existing SSF studies which used lignocellulosic material (Jahromi et al. 2011; Zambare 2010). This is emphasized as fungal biomass in solid-state fermentation is difficult to be quantified as the cells are closely bound to the substrate, thus, making it impossible to measure the cell weight unattached to the substrate (Mitchell et al. 2004). Fungal biomass in this study was indirectly measured based on the formation of glucosamine in fungal cell wall.

Lignocellulolytic enzyme production

Table 3 compares the production of enzyme by different fungal strains when fermented on OPF petiole. All six strains demonstrated the production of enzyme cocktail, consisting of endoglucanase, β-glucosidase, exoglucanase, xylanase, lignin peroxidase, manganese peroxidase, and laccase. However, the production of these enzymes differed in terms of composition and activity. Among them, lignin peroxidase was significantly secreted ($p < 0.05$), followed by xylanase, and cellulase. This is because the lignin and hemicellulose structures are intertwined and are much accessible than cellulose (Sorek et al. 2014), which may have resulted in high induction and secretion of lignin peroxidase and xylanase. Among the strains, *A.niger* EFB1 and *T. virens* UKM1 showed the highest secretion of lignin peroxidase at 215 and 222 IU/g, respectively. Lignin degradation involves at least two of the three ligninolytic enzymes (lignin peroxidase, manganese peroxidase, and laccase). Other strains such as *T. viride* MMS3 and *T. virens* UKM1 dominated the production of manganese peroxidase at 3.3 IU/g and laccase at 8.3 IU/g. Due to its high redox potential, lignin peroxidase is the primary catalyst in the de-polymerization

Table 3 Lignocellulolytic enzyme activity of different filamentous fungi

	T. asperellum MR1	*T.virens* UKM1	*T.viride* MMS3	*A. niger* EFB1	*A.awamori* MMS4	*A. fumigatus* SK1
Endoglucanase (IU/g)	1.53 ± 0.50	1.57 ± 0.45	2.49 ± 0.57	9.35 ± 0.50	9.06 ± 0.41	5.31 ± 0.55
β-glucosidase (IU/g)	0.43 ± 0.65	0.61 ± 0.90	0.85 ± 0.69	1.21 ± 0.79	1.40 ± 0.89	0.94 ± 0.83
Exoglucanase (IU/g)	0.72 ± 0.70	0.65 ± 0.77	0.88 ± 0.60	1.14 ± 0.54	1.07 ± 0.80	1.28 ± 0.80
Xylanase (IU/g)	5.69 ± 0.96	6.02 ± 0.71	8.28 ± 0.64	106.37 ± 0.57	109.08 ± 0.63	34.59 ± 0.70
Lignin peroxidase (IU/g)	30.85 ± 1.84	222.30 ± 2.70	108.54 ± 1.03	214.49 ± 1.52	164.15 ± 2.75	20.27 ± 1.29
Manganase peroxidase (IU/g)	0.71 ± 0.81	0.82 ± 0.89	3.30 ± 0.85	0.18 ± 0.82	0.21 ± 0.81	0.71 ± 0.71
Laccase (IU/g)	3.08 ± 0.75	8.30 ± 0.92	2.30 ± 0.66	0.97 ± 0.90	1.03 ± 0.92	3.13 ± 0.68

IU/g represents enzyme unit per gram solid

The enzyme activity represents the average 7-day activity

All values are average ± standard deviation of mean from triplicate sample

of non-phenolic lignin (Abdel-Hamid et al. 2013), which explains the high production of lignin peroxidase in all strains tested compared to manganese peroxidase and laccase (Table 3). Despite this, soft-rot fungi are not effective lignin degraders as compared to white-rot fungi. A study using *Pycnoporus sanguineus* was capable of producing much higher laccase (up to 45.6 IU/g) when fermented on OPF (Vikineswary et al. 2006). Among the soft-rot fungi, only *A. niger* and *T.viride* were reported to possess good lignin degrading capability (Satyanarayana and Johri 2005).

Different enzymes in a cocktail influence each other differently. For instance, the high secretion of lignin peroxidase in *A.niger* EFB1 and *A. awamori* MMS4 could have induced the reported high production of xylanase (106–109 IU/g) and cellulase (11.7–11.5 IU/g), while the high secretion of laccase in *T.virens* UKM1, *T. asperellum* MR1 and *A. fumigatus* SK1 may have resulted in the low production of xylanase and cellulase. Kaya et al. (2000) reported that low-to-moderate levels of non-phenolic lignin degradation increase the production hydrolytic enzyme, particularly xylanase. Laccase meanwhile is involved in phenolic degradation, triggering an increase in phenolic inhibitors, such as ferulic acid, *p*-coumaric acid, and tannic acid in crude enzyme (Oliva-Taravilla et al. 2015). Cellulase are vulnerable to such phenolic inhibition. Among the *Trichoderma* species, only *T. viride* MMS3 indicated high xylanase (8.3 IU/g) and cellulase (4.2 IU/g) production potential. When compared against other strains, these strains exhibited high lignin peroxidase secretions with low laccase.

Furthermore, a distinguishable enzyme production profile between *Aspergillus* and *Trichoderma* species was observed. On average, *Trichoderma* species secreted cellulase with a more balanced composition of endoglucanase, exoglucanase, and β-glucosidase at 57, 23, and 19%, respectively, compared to *Aspergillus*'s composition of 76, 12, and 12%, respectively. This is in agreement with *Trichoderma*'s reputation as a good cellulase producer, added with the fact that efficient cellulose hydrolysis requires a synergistic action of all three cellulolytic enzymes (Singhania et al. 2010; Bhat 2000), while *Aspergillus* species are more capable of producing xylanase, attributed to its capability in producing accessory enzymes, such as pectin, esterase, and amylase, that aid in the breakdown of hemicellulose–lignin crosslink (Gottschalk et al. 2013). In contrast, *Trichoderma* species are more oriented towards lignin degradation based on their higher production of ligninolytic enzymes, such as lignin peroxidase. In this light, lignin degradation is an oxidative process, and SSF generally operates under minimal moisture condition that promotes such degradation. In addition, *Trichoderma* species are known for their high water requirement (Cavalcante et al. 2008), and hence, under minimal moisture condition, they secrete more oxidative than hydrolytic enzymes (Zhou et al. 2015). Thus, based on the enzyme production, it can be said that *Aspergillus* species such *as A. awamori* MMS4 and *A.niger* EFB1 are better suited for the production lignocellulolytic enzyme using OPF petiole under the present SSF condition.

Sugar profile

Figure 4 compares the different concentrations of reducing sugar and polyoses in all six crude extracts. HPLC analysis identified the presence of five mains sugars, namely glucose, xylose, arabinose, cellobiose, and fructose. The varying concentrations of these sugars indicate distinctive hydrolysing and consumption capacity of each fungus during fermentation (Öhgren et al. 2006). Both *T. virens* UKM1 and *A. fumigatus* SK1 showed high concentration of reducing sugar at 2.0–1.4 g/L with prominent presence of arabinose. This shows that the strains are mainly involved in side-chain hemicellulose hydrolysis at the arabinoxylan region. Similar high production of arabinose was also produced by *A. fumigatus* SK1, as reported by Ang et al. (2013), when fermented on oil palm trunk (OPT). In other strains, the concentrations of reducing sugars were lower in the range 0.6–0.8 g/L, which suggest higher consumption of reducing sugars to assist growth. Both *A. awamori* MMS4 and *A.niger* EFB1 detected glucose and xylose in their crude mixture, which indicate a complete hydrolysis of cellulose and hemicellulose. The presence of cellobiose in *A. fumigatus* SK1, *T. asperellum* MR1, and *T. viride* MMS3, on the other hand, indicates an incomplete hydrolysis of cellulose (Rosagaard et al. 2006). The high presence of cellobiose in these strains can be traced to their low production of β-glucosidase, as observed earlier in Table 3. Among the sugars produced, fungi generally prefer to feed on hexose sugar such as glucose as they are much easier to ferment compared to pentose sugar such as xylose (Goswami and Kreith 2007). This is

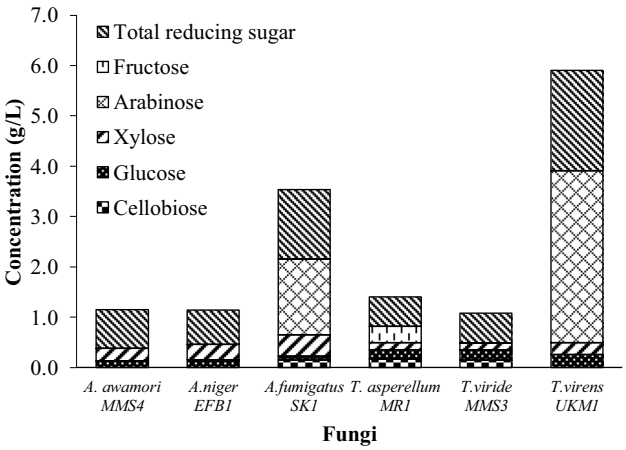


Fig. 4 Sugar profile in crude extract

likely the reason trailing the lower glucose concentration than xylose in all crude extracts.

FT-IR analysis

FT-IR analysis was performed to examine changes in functional groups of the fermented OPF. The transmittance was measured at broad range between 3700 and 800/cm. Changes in peak shape as well as reduction/increment in intensity were observed between non-fermented (control) and fermented OPF petiole spectrum, as depicted in Fig. 5. Changes in alcohol, phenol, alkyl, and aliphatic groups are similar to those reported in the existing OPF fermentation studies (Sharma et al. 2016; Yuliansyah and Hirajima 2012). To better examine the degree of degradation that has taken place, ratio of intensities is compared in Fig. 6. One of the important observations is the reduction in cellulose crystallinity ($I_{900/1098}$). Since cellulose is hard to reach, a strong reduction in cellulose crystallinity suggests effective degradation. In this case, *A. niger* EFB1 and *A. awamori* MMS4 had the highest reduction (75–95%) of cellulose crystallinity in line with its high production of cellulolytic enzymes. *A. niger* EFB1 and *T. virens* UKM1 showed a significant reduction in lignin related linkages such as lignin/hemicellulose ($I_{1511/1738}$) and cellulose/lignin ($I_{900/1511}$), which can be attributed to their high secretion of ligninolytic enzyme. Furthermore, both strains were the only sampled fungi to exhibit lignin reduction at guaiacyl and syringyl ($I_{1268/1244}$)

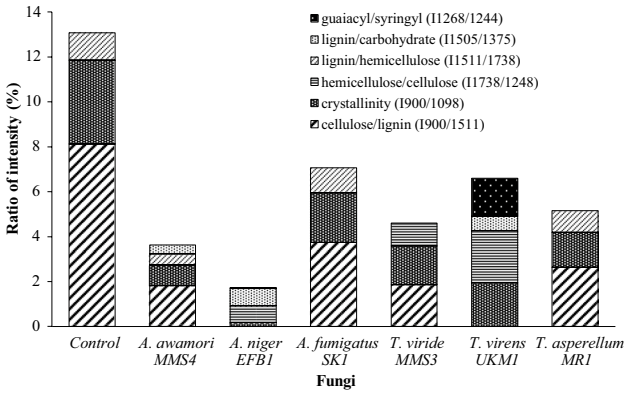


Fig. 6 Intensity ratio of fermented petiole fiber. Wavenumber and assignments: 900 cm^{-1} – amorphous cellulose, 1098 cm^{-1} – crystalline cellulose, 1244 cm^{-1} – C–O–H deformation, and C–O stretching of phenolic, 1375 cm^{-1} – C-H stretch in cellulose (Corredor et al. 2009) | 1248-1268cm^{-1} – Guaiacyl ring breathing with –CH deformation in hemicellulose, 1738 cm^{-1} – C=O stretching in hemicellulose (Bodirlau and Teaca 2009; Kline et al. 2010), and 1504–1515cm^{-1} – C=C aromatic in lignin (Lai and Idris 2013)

levels. The higher laccase produced by *T. virens* UKM1 may have resulted in a higher guaiacyl and syringyl ($I_{1268/1244}$) intensities compared to *A. niger* EFB1. Such lignin degradation would reduce aliphatic and aromatic cross links with hemicellulose, which in turn exposes more hemicellulose for xylanase hydrolysis, hence, contributing to the high production of xylanase for both strains in their respective genera

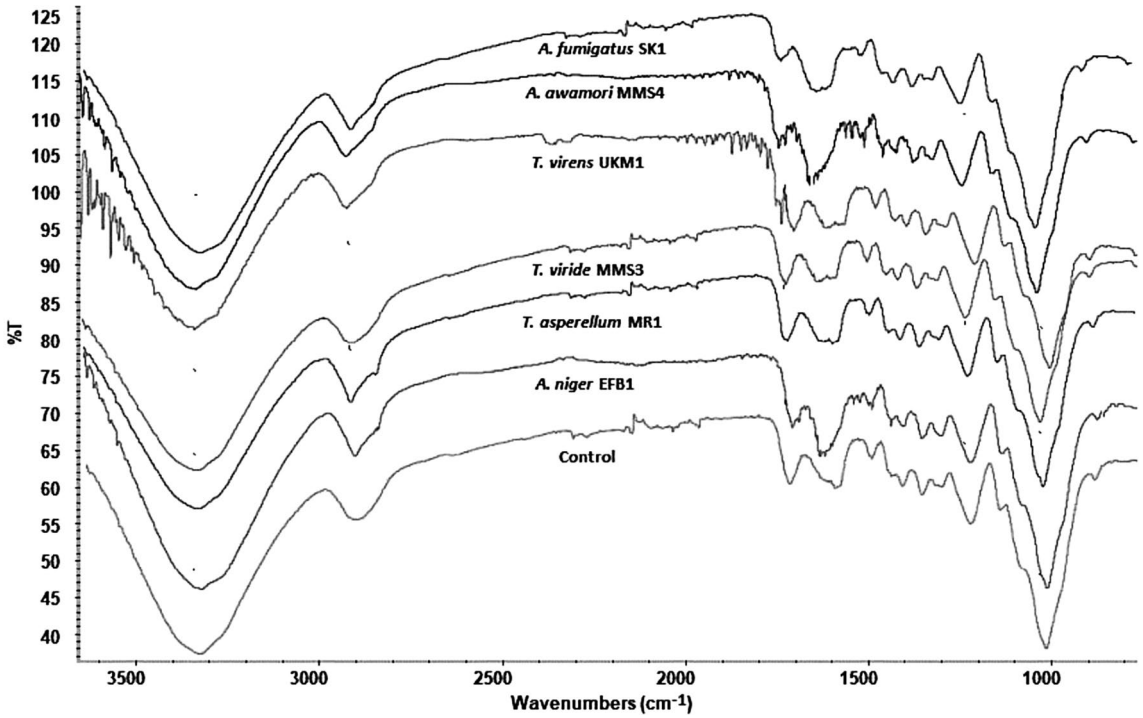


Fig. 5 FT-IR spectrum of non-fermented (control) and fermented petiole fiber with a scanning range of 800–3800 cm^{-1}

(Table 3). Poor performing strains, such as *T. asperellum* MR1, *T. viride* MMS3, and *A. fumgiatus* SK1, demonstrated similar low intensities of lignin, cellulose, and hemicellulose degradation. The FT-IR findings confirm *A. niger* EFB1 and *A. awamori* MMS4 as the ideal candidates to be used in OPF petiole degradation under solid-state fermentation based on their higher lignocellulose degradation capabilities.

Conclusion

OPF petiole was characterized and fermented to study the enzymatic potential under solid-state fermentation. Removal of OPF leaflets and stems reduced lignin content while benefiting soil nutrient recycling. Physical pre-treatments such as pressing had led to a significant reduction of OPF's high free sugar concentration that inhibited growth, while sizing and autoclaving reduced the presence of silica on fiber surface. The results confirm that the combination of 60% moisture content and 0.125 mm particle size provided a sufficient surface area, porosity, and density in facilitating optimal fungal biomass growth. Furthermore, fermentation of frond petiole resulted in multi-enzyme mixture production, which are high in lignin peroxidase and xylanase, alongside endoglucanase, exoglucanase, and β-glucosidase. Both enzyme profile and FT-IR analysis demonstrated the beneficial role of lignin peroxidase in improving xylanase and cellulase production. Finally, compared to *Trichoderma* species, *Aspergillus* species such as *A. niger* EFB1 and *A. awamori* MMS4 produced higher lignin peroxidase and xylanase, suggesting superior capabilities in degrading OPF petiole under SSF condition.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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