

BIODEGRADATION STUDY OF *PYCNOPORUS SANGUINEUS* AND ITS EFFECTS ON STRUCTURAL AND CHEMICAL FEATURES ON OIL PALM BIOMASS CHIPS.

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Pycnoporus sanguineus is a white rot fungus that is known for selective and destructive wood bio delignification in tropical forests. This filamentous fungus is harvested on oil palm biomass (OPB) under solid substrate fermentation (SSF) and produces white-rot decay by generating ligninolytic enzyme Laccase (Lac). Laccase is produced at room temperature in the presence of Kirk's media supplemented with glucose, ammonium nitrate and corn steep liquor. The Lac enzymes are able to endure temperatures ranging from 10 to 70°C and also the pH variation from 2.5-6. The synthesis of this enzyme, accountable for lignin degradation in oil palm biomass can further be exploited to degrade the unmanageable organic contaminants in the atmosphere. It is observed that selective lignin degradation was produced by *P.sanguineus* for time period of 4 weeks. The weight and component losses of oil palm trunk chips after decay were evaluated and analyzed by Scanning Electron Microscope. The maximum Laccase activity was 1007.8 U/L on the 10th day of incubation. The present work is dedicated to assess the biodegradation of oil palm biomass and study for its pretreatment of enzyme activity.

Keywords: Fungal pretreatment; Lignocellulose; Pycnoporus sanguineus; Laccase

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INTRODUCTION

Lignocellulosic wastes are composed of cellulose, hemicellulose and lignin which are the most important part of the natural resources that are discharged by the farming and agricultural activities (Dashtban et al. 2009; Sanchez 2009). Lignin is an obstinate and aromatic, three dimensional macromolecular compound, made up of phenyl propane entity that is intimately linked through carbon-carbon bonds (Mahajan 2011) to defend the plant structural polysaccharide against the microbial attack (Gellerstedt and Henriksson 2008; Sánchez 2009). White-rot fungus (WRF) has been broadly studied for their skills to biodegrade lignin and carbohydrates (Higuchi 1985; Mendonca et al. 2002). They are the sole microorganisms on terrestrial ecosystems capable of decaying wood via secretion of nonspecific hydrolytic and oxidative ligninolytic enzymes, namely lignin peroxidases (LiP), manganese peroxidases (MnP) and Laccases (Lac) (Eriksson and Lindholm, 1971; Halis et al. 2012) and also low molecular mass compounds that

mediate the action of these enzymes. The extracellular enzymes are crucial for depolymerization of cellulose and degradation of lignin (Akhtar et al. 1997). White-rot fungus can be employed for many biotechnological purposes, like in the pulp and paper industry for pitch control, biopulping and biobleaching (Akhtar et al. 1998). They are beneficial to the environment and industries due to their capability to decompose a lot of organic pollutants, xenobiotic compounds, chlorinated phenolics (Elisashvili et al.; Roriz et al. 2009) polycyclic aromatic hydrocarbons (Cho et al. 2002) and simultaneously for the efficient bioconversion of plant and agricultural residues (Mendonça et al. 2002).

There is description of nearly 10,000 species of WRF, having different aptitude for depolymerization of lignocellulosic materials and further decay of lignin to carbon and water (Kirk 1984; Yaver et al. 1996; Highley and Dashek 1998; Halis et al. 2012), though the actual may be around 5 million species (Blackwell 2011). Wood-rotting fungi play a central role in ecology of forest ecosystems by maintaining the carbon cycle by plant photosynthesis (Martinez et al. 2011). Biopulping is the fungal pretreatment of wood with white-rot fungi that aims to make possible fiber separation and lignin removal in mechanical or chemical pulping process (Akhtar et al. 1998; Singh et al. 2010). It has been demonstrated that this biotreatment is advantageous, as consumption of energy during defibration and refining is lesser, delignification rate is faster, and overall improvement of pulp strength properties is observed (Singh et al. 2010). *P. sanguineus* is a cosmopolitan white-rot fungus of significance, as it has a budding capacity to be multipurposely used in biotechnological methods (Talaiepour et al. 2010). Because of its capability to degrade lignin aggressively, it can also be a part of the lignocellulosic feedstock biorefinery (Lomascolo et al. 2011). Laccase belongs to the group of enzymes that are called blue multi copper oxidases, which have broad substrate specificity (Baldrian 2005; Shradha et al. 2011). The chief functions of Laccase in fungi and higher plants are to perform morphogenesis, pathogenesis and sporulation (Dwivedi et al. 2011; Babu et al. 2012). Additionally the enzyme helps in decomposition of lignocellulosic materials and in the oxidation of diverse aromatic compounds (Thurston 1994; Gianfreda et al. 1999; Renuka et al. 2011).

Oil palm biomass (OPB) is a surplus agricultural by product generated from oil palm (*Elaeis guineensis*) tree in Southeast Asia, which comprises of 80% of world oil palm plantations (Sulaiman et al. 2012). Oil palm is a perennial crop and gives us valuable palm oil that is consumed as edible oil and also has many industrial uses (Malaysian oil palm board statistics, 2008). As the commercial utilization of oil palm has increased, the amount of residues in form of empty fruit bunches; fronds and trunk are generated equally. Singh et al. (2011) concluded that one hectare of oil palm cultivated area generates 50–70 tonnes of biomass residues. The Malaysian oil palm industry currently makes the largest amount of biomass in country with 30 million metric tons accounting for nearly 85.5% of the nation's overall wastes (Shuit et al. 2009). Amongst OPB, oil palm trunk (OPT) is a cheap lignocellulosic material which is easily obtainable, thus it makes good sense to fully exploit its potential (Bahrin et al. 2012; Sulaiman et al. 2012). Using oil palm trunk for pretreatment can reduce the load on the forests and preserve biodiversity providing us economic gain in turn (Singh et al. 2012). The rate of biodegradation of the biomass residues is regulated by various aspects such as

temperature, moisture content, and type of lignocelluloses (Tuomela 2002). Microorganisms that colonize and biodegrade do not gain energy from the white-rot decay, but assist in competent use of carbohydrates. *P.sanguineus* is a microorganism to be noticed for their capability in the pretreatment of lignocellulosic biomass that is the most central step needed for eradication of lignin. Its remarkable skill to degrade the lignin content of wood pulp is the characteristic feature that holds promise for the paper pulp industry, as this would cut the use of chlorine in bleaching and thus reduce environmental contamination utilizing plant residues (Lomascolo et al. 2011). Several researchers have mentioned that, as the fungi eliminate lignin carefully without appreciable losses of cellulose, thus have high potential for use in biological pulping processes (Blanchette 1995; Akhtar et al. 1998). For the production of enzymes biological pretreatment are considered superior to chemical processes as product yields are higher, with less energy demand and the reaction conditions are environmentally benign (Yu et al. 2010).

The aim of present work was an attempt to use in vitro laboratory tests to estimate the ability of *P.sanguineus* for laccase activity using Solid state fermentation (SSF) and to know the outline of decay produced by fungi using scanning electron microscopy (SEM). In SSF the microorganisms are cultivated on humid solid substrates making use of water insoluble lignocellulosic material for microbial growth. Thus this method is considered to be most suitable for Basidiomycetes fungi cultivation as they nurture well closer to their natural habitations (Pandey et al. 1999, Leonowicz et al. 2001; Nyanhongo et al. 2007; Brijwani et al. 2011). Based on the above mentioned and the hopeful observation of applying laccase for biotechnology, in this work we examined the biodegradation pattern of *P.sanguineus* by solid state culture.

EXPERIMENTAL

Materials

The white rot fungi *Pycnosporus sanguineus* KUM 70097 pure cultures was kindly provided by Forestry and Forest Products Research Institute (FPPRI), Japan. Stock cultures were maintained at 4°C on malt extract (2%) and potato dextrose agar (PDA) slopes (Difco) with periodic transfer. The fungus was identified as one with ligninolytic potential, as a visible clearance of agar plates was seen when glucose 0.2% (w/v), mycological peptone (Oxoid) 0.01% (w/v), yeast extract (Difco) were added in the medium.

Growth Conditions

Inoculum preparation: In 100 mL of the Kirk's modified media added 10g/L glucose, 2 g/L ammonium nitrate, 2 g/L corn steep liquor (CSL) and 2 g/L yeast extract. Later 20 mycelial discs (0.5cm, diameter from 7 day culture) from potato dextrose agar (PDA) were taken and incubated for 7 days with continuous vibration. After the fungi had grown completely on the medium surface, the mycelium mat was gathered and rinsed carefully with distilled H₂O. Later it was homogenized in a sterile waring blender for 15 secs with 100 mL distilled H₂O and 500 g (dry basis) of sterile OPT chips were added

into each flask. Sterilization of these chips was achieved by pre-soaking in H₂O for nearly 10 hrs followed by excess water drainage and then autoclaving the humid chips at 121°C for 15 min. Fungal pretreatment was done in 2000 mL Erlenmeyer flask using the chips of size (2.5cm x 1.5cm x 0.2cm) from the inner part of OPT log. Moisture content was made up to 55% to make certain an optimal colonization and penetration of fungal hyphae (Ferraz et al. 2008). The flask with OPT chips served as untreated controls (C). Cultures were grown in triplicates and incubated at 25 ± 3°C. The samples from the flasks were harvested every second day after the pre-treatment and enzyme activity tests were done. Biodegraded chips were further analyzed through scanning electron microscope (SEM). The morphology and the condition of inner matrix cells of OPT chip were examined using SEM (LEO Supra 50 Vp, Carl Zeiss SMT, and Germany). Micrographs were taken from the cross section of the samples with dimension of 0.5 cm x 0.5 cm and were coated with gold by an ion sputter coater (Model Polaron SC515, Fisons Instruments, and UK).

Enzyme Assays

Extraction buffer

Sodium acetate 50mM (pH 5.5) supplemented with 0.1 g/L Tween 85 (Sigma Aldrich) was used to extract enzyme, the chips are recovered and extraction were done with 500 mL buffer using a magnetic stirrer with shaking at 2500 rpm for 10 min at 4 ± 2°C (DeSouza Cruz et al. 2003). The crude extract prepared was recovered by filtration through Whatman No.1 filter paper and the supernatants were used for enzyme activity measurement. The spectrophotometric method with UV-Lambda 160A Perkin Elmer USA has been used to find out the enzyme activity in the experiments.

Laccase (Lac) activity was determined by method of Bourbonnais et al. (1998). The oxidation of 1 mM of 2, 2'-azino-bis (3- ethylbenzthiazoline-6-sulfonic acid (ABTS) was measured by rate of formation of the cation radical ($\epsilon_{420} = 3.6 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) in acetate buffer (pH 4.5). The supernatants were prepared by filtering the mycelium and then centrifuging the filtrate for 20 min at 5000g. Reactions were done in 3 mL cuvette with 0.6 mL of buffer, 0.2 mL H₂O, 1 mL of enzyme extract and 0.2 mL of 1mM substrate. Absorbance was read at 420 nm against a suitable blank within 3 mins of reaction. The formula used to calculate activity is:

$$\text{Activity of laccase} = n \times \frac{\Delta A \times 106}{23,300 \times 3}$$

One unit of laccase activity is defined as the amount of enzyme required to oxidize 1 μmol of substrate (ABTS) per unit of time (min).

Weight Loss

Prior to incubation chips were dried to constant weight at 40°C, after treatment chips were washed using distil water and further filtrated to eradicate the microorganisms and components. These chips were dried at 40°C for 48 hrs and loss of weight was calculated based on the initial and final dry weight as described by Talaeipour et al. 2010.

Lignin determination

The pre treated chips were taken out after 4 weeks of incubation and then pulped mechanically using refiner in accordance with Tappi methods. Lignin and Cellulose was determined as described in Tappi 222 om-88 and Tappi 452 om-88.

RESULTS AND DISCUSSION**Effect of Carbon source on Laccase Activity of *P.sanguineus***

Oil palm biomass contains 10-15% of reducing sugars and nearly 30-35% of soluble carbohydrates (Akmar and Kennedy 2001; Hashim et al. 2011), thus we presumed that the extractable carbohydrates from oil palm trunk may perhaps augment the ligninolytic enzyme production. Fungal pretreatment and ligninolytic enzyme cultivation on OPT substrate under solid state fermentation have been reported for the very first time. Therefore the determination of ideal parameters will be a milestone towards knowing the optimum condition for biotreatment of OPT. The ligninolytic enzyme produced by *P. sanguineus* in Kirk's modified nutrient medium containing glucose as the carbon source, ammonium nitrate as nitrogen source, CSL as a fungal growth promoter were observed. Previous studies conducted on the fungi conclude that Kirk's nutrient media is appropriate for studying ligninolytic and Lac activity (Calvo et al. 1998; Eugenio et al. 2009). The research also emphasized that the growth of fungi and enzyme produced highly depends on the method of fungi cultivation (Elisashvili et al. 2008). The key factors for optimum growth are sources of carbon and nitrogen, temperature and pH. Moderately high level of carbon and low nitrogen level is needed for fungal growth in WRF and it has been stated that low nutrient nitrogen level is often a prerequisite for lignin degradation (Eriksson et al. 1990). Biotreatment under the most favorable condition allows the fungus to grow quickly, in fact it is visible, that the SSF support ample amount of volumetric laccase activity. The results of enzyme activity indicates that Lac activity increases gradually over the first few days of incubation, then peaks and further declines continuously (Figure 1). The highest Lac activity (1007.74 U/L) was obtained on the 10th day of incubation (Figure 1). The results are in agreement with the earlier studies concluding that glucose is the best carbon source, for the Lac activity. Stajić et al. (2006) analyzed many other polysaccharides (fructose, maltose, cellulose) as carbon sources during pretreatment with *Pleurotus eryngii* 616 and *Pleurotus ostreatus* 493 and 494 cultures, and accounted that laccase activity was maximum when glucose is supplied, than in any other carbon source in the media. Mikiashvili et al. (2006) obtained comparable results using *Pleurotus ostreatus* 98 in biodegradation culture. Lee et al. (2006) tested *Trametes versicolor* KCTC 16781 cultures and achieved highest Laccase activity in media with glucose in comparison to when fructose and sucrose are supplemented. According to Schlosser et al. (1997), *T.versicolor* was cultivated on glucose, wheat straw and beech wood; it produces Laccase both in extracellular and intracellular fractions. Moderately high level of C and low N level is needed for fungal growth although some wood-rotting fungi, low nutrient nitrogen level are often a prerequisite for lignin degradation (Eggert et al. 1995; Dix and

Webster, 1995). Maximum enzyme activity was achieved at day 10 (1007 mU/ml), although considerable activity levels (738.10 mU/ml) were detected up to day 14.

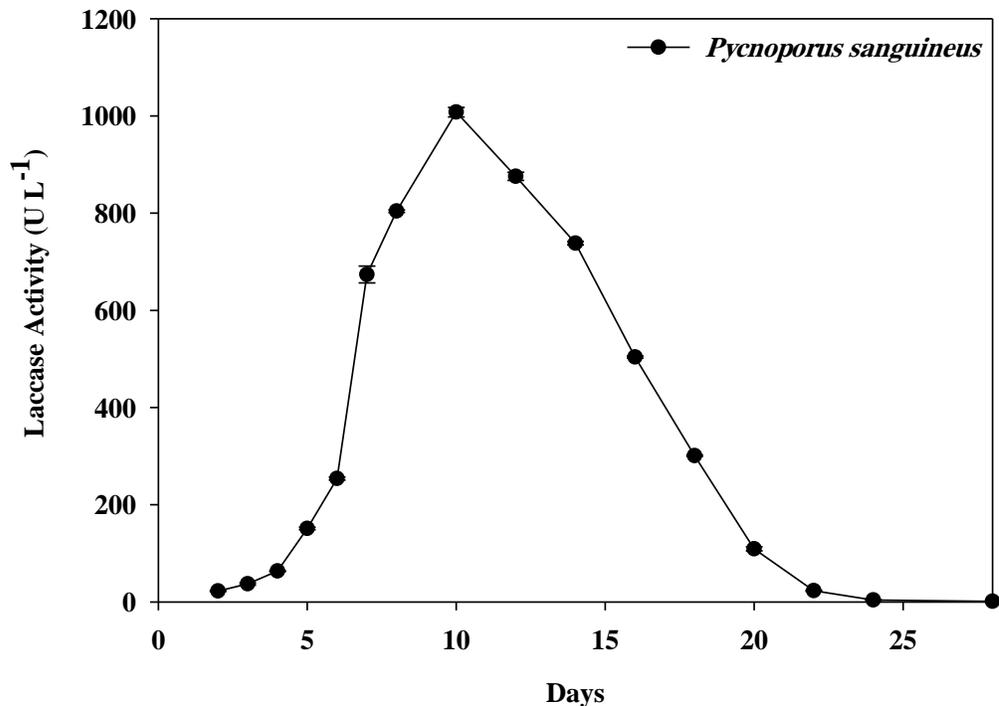


Figure 1. The production of Laccase by *P. sanguineus* over incubation periods varying from 1 to 28 days. The data were mean of the values obtained from triplicates data and standard error is calculated.

The Effect of Temperature on Laccase Activity

Temperature is one of the most important factors affecting fungal growth though there are no universal conditions (Mayer and Staples 2002; Wanqing et al. 2011). Temperature profiles of laccase activity usually tend to be similar to the other extracellular ligninolytic enzymes with optima between 25°C and 60°C. The most favorable temperature of Laccase can fluctuate greatly from one strain to another, but fungi are unable to grow at the very high temperatures. Litthauer et al. (2007) reported that *P. sanguineus* is capable of manufacturing laccases at enduring temperatures of 60–75°C for more than 1 hr. Temperature range of 10°C to 70°C was examined, by incubating the assay reaction at various temperatures for 10 min. Farnet et al. (2000) found that the laccase isolated from a strain of *Marasmius quercophilus* were stable for 1 hr at 60°C, they later found that pre-incubation of enzymes at 40 and 50°C amplified laccase activity to a large extent. As the enzymes in biological by-product are generally thermo labile, it is observed that under influence of heat, the enzymatic hydrolysis of pretreated OPT decreases significantly, Zhang et al. (2011) illustrated that the enzymes in the byproducts were the main factors enhancing the subsequent hydrolysis of *Irpex lacteus*, corn stalks. Nyanhongo et al. (2002) showed that laccase generated by *Trametes modesta* was fully active at 50°C. The thermo stability of the enzyme in present

experiment was assessed by estimating the laccase activity every 10 min for 80 min using ABTS as substrate at optimal pH over a temperature range of 30°C to 70°C.

Enzymatic temperature stability

The amount of purified laccase activity remaining following incubation at temperatures ranging from 30 to 70°C was measured during the times stated. 1 mM ABTS in 100 mM acetate buffer (pH 4.5). The temperature dependence of activity of free enzyme is shown in Figure 2. For thermal stability studies, the enzyme was pre-incubated in 50 mM citric acid buffer (pH 3.0) at 10–70°C for 0–240 min, and the residual enzymatic activity against ABTS was then determined at the optimal pH (3.0) and temperature (50°C). Laccase exhibited maximal activity at 35°C though it grows at the elevated temperatures (47°C) and salt concentrations (500 mM). Ko et al. (2001) reported the laccase enzymes from *Ganoderma lucidum* with the highest activity at 25°C with optima below 35°C.

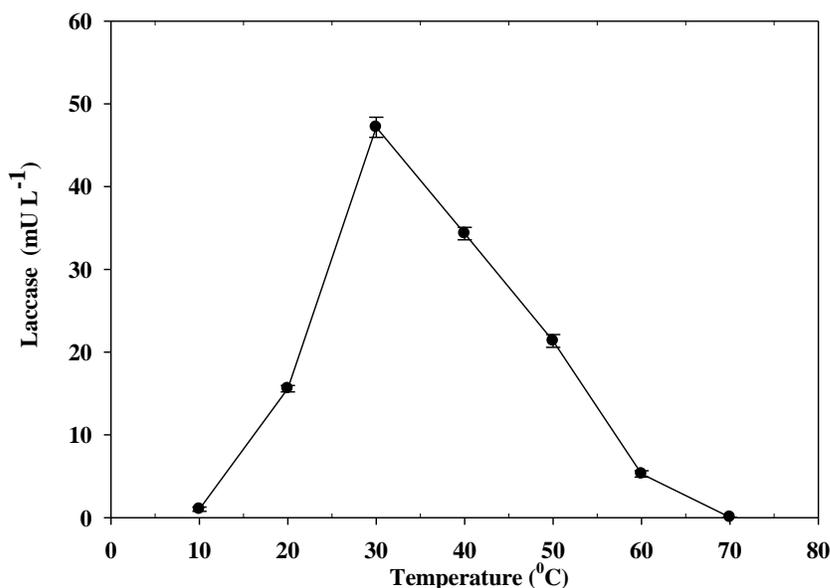


Figure 2. The Laccase activity following incubation at temperatures ranging from 10 to 70°C. Error bars denote the standard deviation of three replicates for each experimental point.

Influence of pH on laccase activity and stability

The enzyme activity was greatly affected by the hydrogen ion concentration of the media (Chen et al. 2002). To determine the optimal pH, laccase activity was measured over a pH range of 2.0 to 7.0 using ABTS as substrate. The pH gradient was obtained by sodium acetate buffer (2.0 to 7.0) and HCl / KCl buffer (1.0). The effect of pH on the stability was determined by incubating the purified enzyme at room temperature at different pH levels for 4 h and calculating the residual activity. The pH optima of laccase are highly dependable on the substrate, as using ABTS the pH optima are more acidic and are found in the range 3.0-5.0 (Kunammenni et al. 2008). The influence of pH on the activity of enzyme is shown in Fig. 4. The figure shows that the most favorable pH range

for the maximum production is 4.5. The Laccase activity profile is almost bell shaped with an optimal pH that varies considerably. The maximum production of enzyme after 2 days at pH 4.5 being 96.8 U/ml followed by pH 4 which was 90.4 U/ml. Previously, Leonowicz et al. (1999) had concluded that fungal laccases are stable at acidic pH. Blaich and Esser (1975) did screening of lac activity among wood-rotting fungi stained with p-phenylene diamine after isoelectric focusing, and all the trial microorganisms demonstrated the production of more than one isoenzyme, with pI (isoelectric point) value in the range of pH 3–5. Thus our observation is consistent with preceding results.

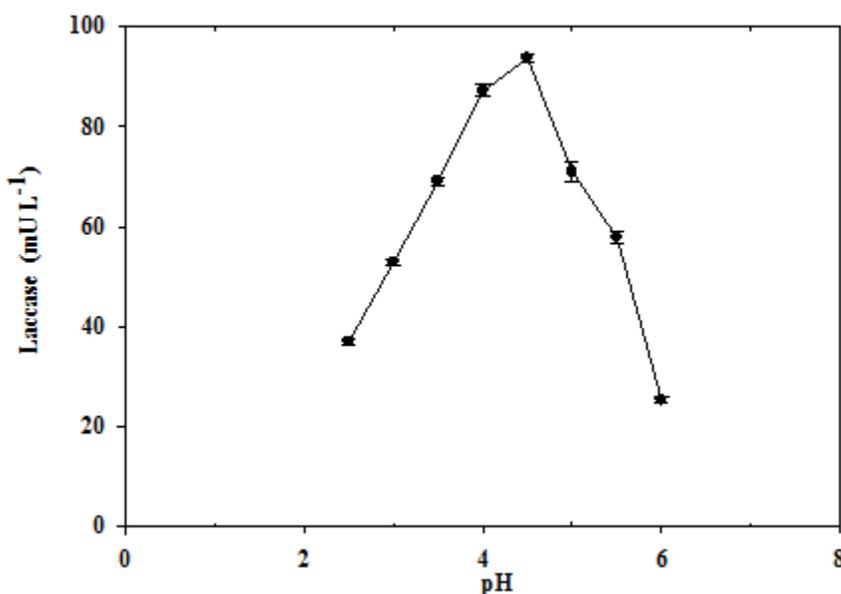


Fig. 3. The effect of pH on activity of Laccase in OPT during pre treatment. The data were mean of the values obtained from 3 samples and standard error is calculated.

Influence of Solid State Fermentation

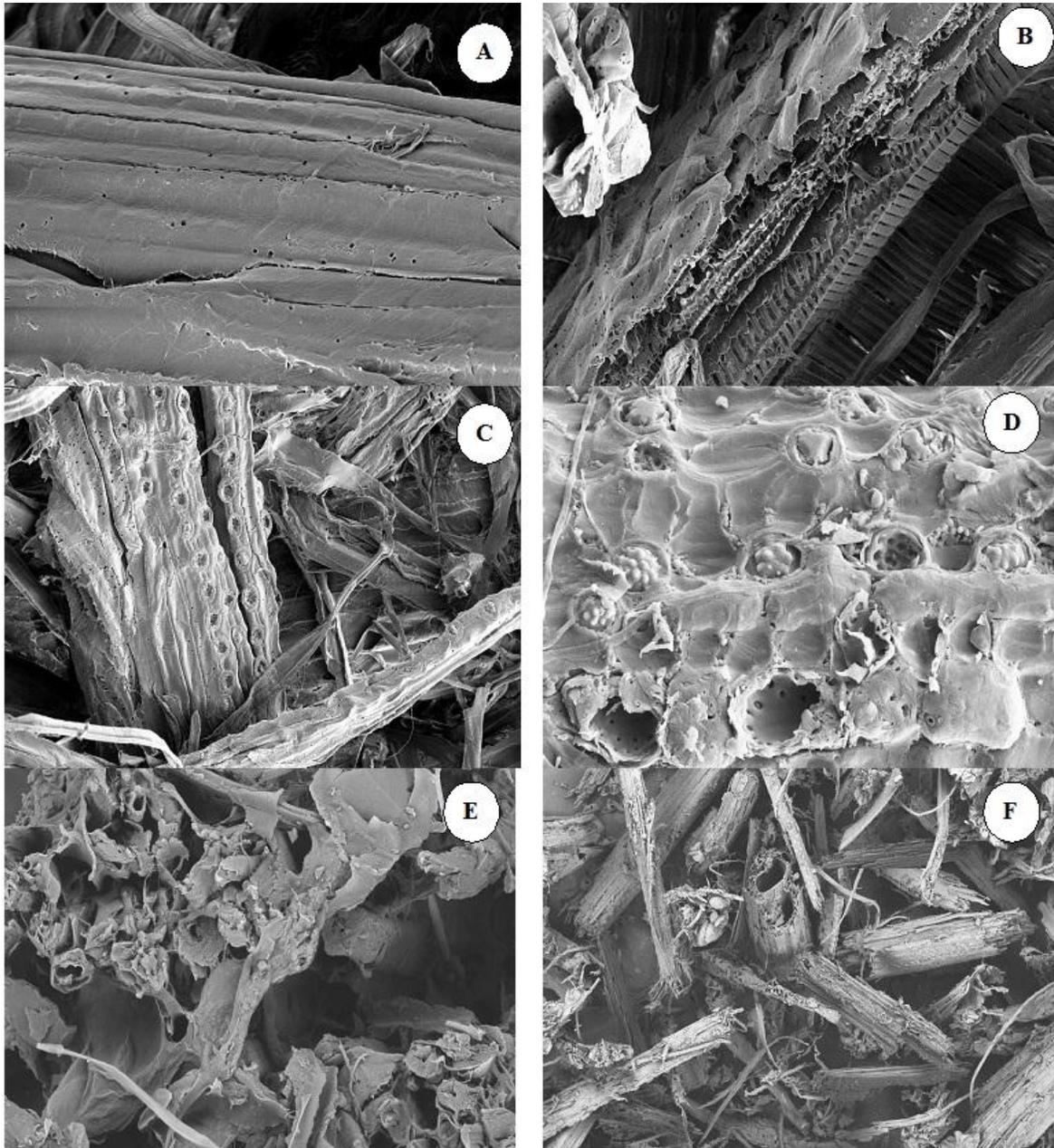
The conditions of the culture medium had a marked effect on Laccase activity, which increased up to 1007 mU/mL that is a 5 fold rise over the control (197.45) mU/ml. The technique of Solid-state fermentation has been used for enzyme production by fungi due to high productivity (Hong et al. 2011). Couto et al. (2009) utilized dye absorbed lignocellulosic substrate and reported high laccase production by *Trametes versicolor* and *Funalia trogii* during solid-substrate fermentation. *Funalia trogii* produced 2.78U/mL Laccase enzyme on day 10 in Astrazon black dye absorbed wheat bran medium for fungal growth. Using *Trametes versicolor* for dye biosorption maximum laccase activity 2.74U/mL was detected on day 10 which declined further. Kadam et al. 2012 reported .34U/mL laccase activity using a consortium of *Pseudomonas* SUK1 and *Aspergillus acheaceus* NCIM 1146 when they used lignocellulosic substrate under solid-state fermentation. They reported that solid-state fermentation is favorable for fungal growth and is competitive procedure in comparison with thermo chemical processes, both in

efficiency and from an environmental safety view (Raimbault 1998). Okazaki et al. (1980) accounted that the α -amylase activity in *Aspergillus oryzae* grown in SSF on steamed rice was in direct proportion to its mycelial weight. Gupta et al. (2011) obtained the biological delignification of lignocellulosic feed stocks from *Prosopis juliflora* and *Lantana camara* with *Pycnoporus cinnabarinus* under SSF conditions with a pre-treatment scalability up to 500 g of substrate. Currently SSF has been applied to large-scale industrial processes largely for the production of hydrolytic enzymes. The filamentous fungi are capable to penetrate into solid substrates as the hydrolytic enzymes excreted from the hyphae increases the accessibility of all available nutrients within particles (Raimbault 1998).

Scanning Electron Microscopy (SEM)

The biodegradation pattern of the white rot fungi on OPT chips, for time duration of 0 to 28 days, is shown in Figure 4. It is observed from SEM picture that the lignin removal is restricted to surface areas of the decayed wood chips (Ferraz et al. 2002). *P. sanguineus* degraded lignin selectively, as the lignin losses were higher than the cellulose loss (Table 1). Akhtar et al. (1998) concluded that selective lignin degradation is essential in a pretreatment method as fungal species can be selected based on their ability to degrade. Although the extent of lignin degradation in fungal pre treatment of wood material is not directly related to the benefits found in biomechanical and fungal-organosolve pulping. SEM micrographs revealed that white pockets are formed, but are restricted to superficial areas, it highlights that there is careful lignin removal, during pretreatment (Ferraz et al. 2003). The fungi deconstructed lignin in the initial period of pre treatment and leave cellulose unbroken to some extent. The improvement of enzymatic hydrolysis during biological pre-treatment has been attributed to lignin degradation (Sun and Cheng, 2002). It can be concluded that white-rot fungi *P. sanguineus* favours the biological pre-treatment of oil palm biomass and has a great deal of interest as biomass decomposers for large scale applications due to their ability to produce large amounts of extracellular lignocellulolytic enzymes. Silica bodies were present extensively on the strand, and are attached to the circular craters that are distributed uniformly. Effective pretreatment is needed to reduce the lignin content and crystallinity of the cellulose, and to increase the surface area pore size of materials. Since lignin makes the access of cellulolytic enzymes to cellulose difficult, its removal is necessary prior to the enzymatic hydrolysis (Sun and Cheng, 2002).

Figures



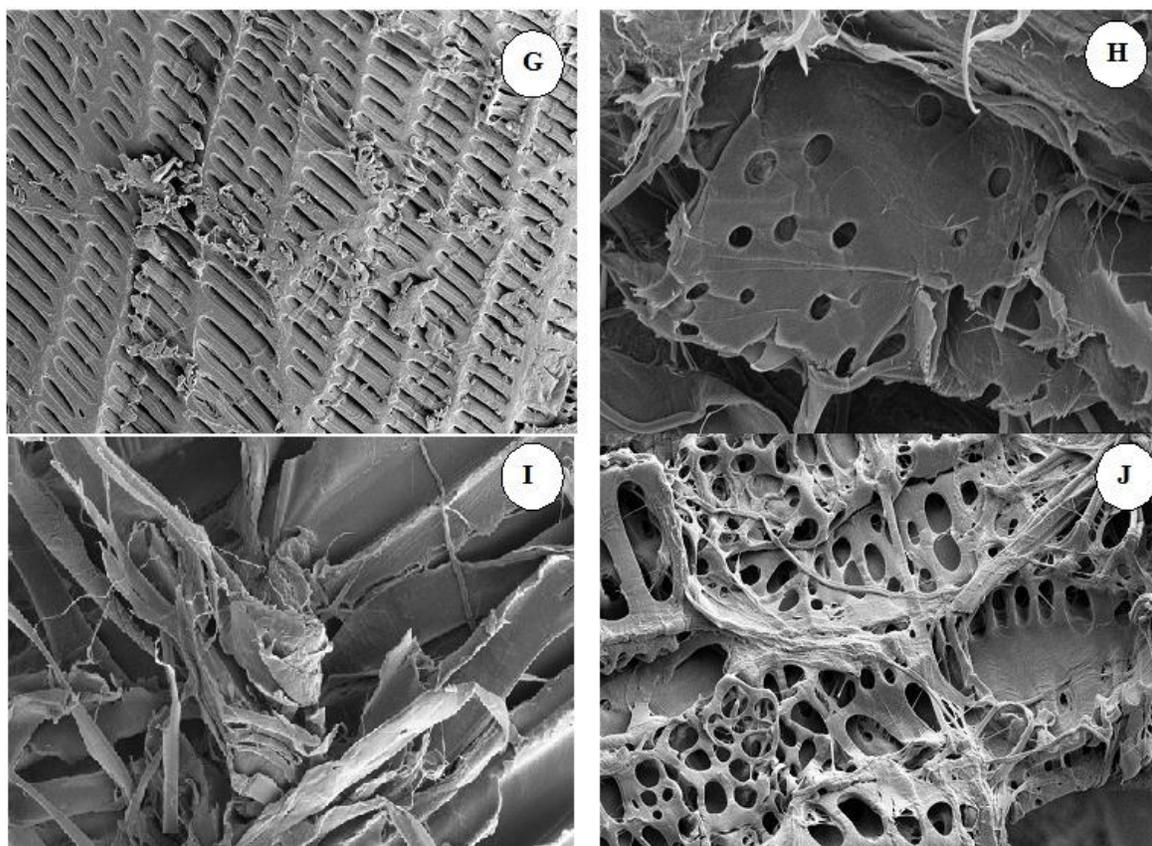


Figure 4. Microscopic structure of *P. sanguineus* treated on OPT chip. The biodegradation pattern of treated OPT with fungus from day 1 of incubation till 28 days.

- (A) Picture of C sample 1 the OPT cell walls are intact, vascular bundles are visible, cellular matrix is intact.
- (B) Day 14 cell walls have started degrading, lignin seems to be getting modified, so as to reduce the chance of attack by carbohydrate. The silica bodies are seen embedded in the OPT structure.
- (C) Day 21 demonstrates that the fungus is quite selective; lignin is partially degraded and cell walls are undergoing further decay.
- (D) Day 28 indicates visibility of the cellulose fibrils floating in the matrix. It is clear that silica bodies have been removed from the matrix, their exclusion is important because their presence creates restraints on the accessibility of enzymes.

Lignin Determination:

In this study, the lignin content in OPT chips was determined at 20.15% (w/w). The lignin was reduced by fungal treatments to values of 16.25% (w/w) after bio-treatment with *P. sanguineus* in the OPT chips. Lignin loss (w/w) (7.85%) was higher than that of total weight (6.25%) and cellulose loss (2.78%) after the 28-day fungal pretreatment period (Table 1). Weight loss is considered as an indicator of fungal degradation. Lignin selectivity ratio was found to be 2.83% in pretreated OPT as

compared to the control (Table 1). The selectivity for lignin degradation suggests that species secretes high level of lignin degrading enzymes than cellulose and hemicellulose and is therefore lignin degraders which are suitable for bio delignification process. Copur and Tozluoglu (2007), observed *Brutia* pine chips treated with *Ceriporiopsis subvermispora* that after 4 weeks of incubation the weight loss and holocellulose loss was 6.13% and 12.65% respectively. Comparable results were obtained by Gupta et al. 2012 during bio delignification of *Eucalyptus grandis* chips by *Schizophyllum commune* where the lignin contents reduced from 34.20% to 23.2% and loss of holocellulose was 11.59% over a period of 28 days. In the biotreatment of pine chips with *Ceriporiopsis subvermispora*, a higher degradation of lignin was detected over all the periods of biodegradation than cellulose loss, indicating a selective pattern of decay by these fungi (Ferraz et al. 2008). Maximum lignin mineralization was 11.85% for strains after 7 weeks of incubation (Ferraz et al. 2008).

Table 1. Weight loss and changes in chemical composition of oil palm trunk chips after biological treatment.

Fungi`	Total weight loss %	Lignin loss%	Holocellulose loss %	Cellulose loss %	Lignin selectivity (Lignin/Cellulose) %
Control	1.34	0.71	0	0.42	0.26
<i>P. sanguineus</i>	6.25	7.85	12.65%	2.78	2.83

Table 2. Change in the chemical components after biological pre-treatment with *P.sanguineus*.

Fungi	Acid soluble lignin	Alkali extractive (%)	Ash (%)
Control	1.34	0.50	
<i>P.sanguineus</i>	0.91	16.42	0.35

Values are expressed as percent decrease based on the oven-dried weight of original wood

CONCLUSIONS

1. Results of the study demonstrated that fungal pre-treatment of *P.sanguineus* is successful as fungi was able to grow on oil palm trunk. Therefore Oil palm biomass could be further used as a substrate for fungal growth and laccase production.

2. *P. sanguineus* showed a preferential decay ability to degrade lignin in OPT chips during the incubation period. Selective lignin-degrading fungi are considered better ones as they decrease the lignin content in large amount. Minimal cellulose degradation has also been reported in the current experiment.
3. Laccase generated by the fungus under SSF is easy and affordable; it can be used for novel biotechnological applications. Optimizing the culture medium substantially boosts laccase production.
4. This study indicated that residues of OPT is suitable raw material for the production of high amount of ligninolytic enzyme. This biomass can hold guarantee as an effective solution for some environmental problems, it would be desirable to use them at industrial level also these residues can be a suitable non-wood substitute for pretreatment and delignification..

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