

Biodegradation and ligninolytic enzymes profiles of the newly synthesized organotin(IV)-treated non-durable tropical wood species.

Md. Masudur Rahman, Ismail Jusoh, Ahmad Husaini*, Idris Abu Seman, Ngieng Ngu Sing

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Abstract

The objective of this study was to assess the response of the ligninolytic enzymes production during the biodegradation of organotin(IV) complexes-treated of *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis*. The tropical wood species tested were treated respectively with different levels of monosubstituted organotin(IV) and disubstituted organotin(IV) complexes concentrations (0.1, 0.5 and 1%) and subjected to biodegradation by wood rotting fungi, *Trametes versicolor* and *Gloeophyllum trabeum*. Ligninolytic enzymes profiles of the biodegraded wood showed that manganese peroxidase is the highest enzyme produced and recorded as 26.72 U/mL of the untreated wood and 15.07 U/mL of organotin(IV) treated wood respectively, as compared to lignin peroxidase and laccase. Laccase activity was the least produced among them. Manganese peroxidase is highly expressed indicating that it is most likely to be the predominating enzyme that causing lignin degradation in the biodegraded *A. scholaris*, *M. triloba* and *H. brasiliensis* untreated and organotin(IV) complexes-treated woods. The ligninolytic enzymes activities of dibutyltin(IV)-treated wood were the least determined. Wood densities decreases with the increased in the percentage of weight loss indicating the rapid wood biodegradation occurred. Density reduction of monosubstituted organotin(IV) treated wood was found higher than disubstituted organotin(IV) treated wood. This study shows that the newly synthesized organotin(IV) complexes are effective on reducing the activities of ligninolytic enzyme that plays a vital role in the biodegradation of wood. Dibutyltin(IV) complex is found to affect

the ligninolytic enzymes production and in turn gave the best protection to *A. scholaris*, *M. triloba* and *H. brasiliensis* against the decay fungi tested, *T. versicolor* and *G. trabeum*.

Keywords: Lignin peroxidase; Laccase; Manganese peroxidase; Organotin(IV) complex; Tropical wood

Introduction

Biodegradation is one of the major economic losses to any biological products. Wood products are subjected to various bio-hazard attacks if preventive measures are not taken. Several types of biodegradation have been recognized in wood, are mainly due to fungal decay, bacterial degradation and insect attack. Among all types of biodegradation fungal decay is the most important and widespread type of degradation (Hamed, 2013; Skyba et al. 2013).

Microorganisms have evolved several of enzymes for degrading the different components of lignocellulosic material. These enzymes include cellulases (for degrading the cellulose), xylanases (for degrading the hemicellulose), and ligninolytic enzymes (for degrading the lignin). There are two major families of ligninolytic enzymes which are involve in ligninolysis: peroxidases and laccases (Dominic, 2009; Tanka et al. 2009; Hatakka & Hammel, 2010; Hofrichter et al. 2010). Liew et al. (2011) observed that manganese peroxidase produced is far more superior as compared to lignin peroxidase, suggesting that MnP might be the predominating enzymes causing lignin degradation.

The ligninolytic enzymes attack lignin directly and thereby are the most promising long term biological alternatives to lignin removal by the physical and chemical processes. The ligninolytic enzymes in most basidiomycetes are highly regulated by nutrients such as nitrogen, copper and manganese. Their production is also affected by many typical factors such as medium composition, nature of carbon source, concentration of carbon source, pH of fermentation broth, fermentation temperature, amount and nature of nitrogen source and presence of inducers (Cu^{2+} , Mn^{2+} , 2, 5-xylydine, ferulic acid

Ahmad Husaini*, Md. Masudur Rahman, Ismail Jusoh, Ngieng Ngu Sing

Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan Sarawak, Malaysia.

Tel.: +6 082 583013; Email: haahmad@frst.unimas.my

Idris Abu Seman

Malaysian Palm Oil Board (MPOB), Bandar Baru Bangi, 43000 Kajang, Selangor Darul Ehsan, Malaysia.

and veratryl alcohol) (Asgher et al. 2010; Iqbal et al. 2011; Patrick et al. 2011).

Wood especially the less durable or non-durable tropical species such as *Alstonia scholaris* (pulai), *Macaranga triloba* (mahang) and *Hevea brasiliensis* (rubberwood) must be treated with preservatives in order to protect wood from fungi and insects attack. There is a need to come up with an effective new compound as wood preservative.

Organotin(IV) compounds are chemical compounds based on tin with hydrocarbon substituent. They are an attractive choice and continue to be of interest due to their bioactivities potentials and unique structures.

Previously, the authors has successfully reported on the FTIR spectroscopy analysis and retention of organotin(IV) compounds in *A. scholaris* (pulai), *M. triloba* (mahang) and *H. brasiliensis* (rubberwood) species (Jusoh et al. 2012). As part of ongoing studies on utilizing the organotin(IV) substituted-thiosemicarbazone complexes as wood preservatives, here we report on the effectiveness of these newly synthesized organotin(IV) compounds on reducing and minimizing the production of important ligninolytic enzyme that involved in biodegradation of wood. Thus, the aim of the present study were to determine the main ligninolytic enzyme produced during woods biodegradation and to evaluate the potential of the organotin(IV) complexes as wood preservative with regards to the ligninolytic enzymes profiles generated between the untreated and newly synthesized organotin(IV)-treated *A. scholaris*, *M. triloba* and *H. brasiliensis* that represents the non-durable tropical wood species.

Materials and Methods

Wood cubes, organotin(IV) complexes and treatment.

Three non-durable tropical wood species; *Alstonia scholaris* (Pulai), *Hevea brasiliensis* (Rubberwood) and *Macaranga triloba* (Mahang) were chosen in this study. The logs were quarter-sawed to 25 mm x 25 mm x 25 mm boards and kiln dried. The boards were further planed, ripped and cut into 19 mm cubes according to the AWP standard E10-91 (1991). The wood cubes were then conditioned at 60°C and 70% relative humidity for four days until they reached a constant weight.

Five newly synthesized organotin(IV) compounds were used as the wood preservatives (Affan et al. 2011; Jusoh et al. 2012; Salam et al. 2013). As reported by Affan et al (2011), the reaction of 2-acetylpyridine-*N*(4)-cyclohexylthiosemicarbazone [(HAPCT),(1)] ligand with organotin(IV) chloride(s) afforded the five new organotin(IV) complexes: [MeSnCl₂(APCT)](2), [BuSnCl₂(APCT)](3), [PhSnCl₂(APCT)](4), [Me₂SnCl(APCT)](5), and [Ph₂SnCl(APCT)](6). The ligand (1) and its organotin(IV) complexes (2–6) have been synthesized and characterized by CHN analyses, molar conductivity, UV-vis, FT IR, ¹H, ¹³C, and ¹¹⁹Sn NMR spectral studies. The single crystal X-ray diffraction studies performed showed that [PhSnCl₂(APCT)] (4) was six coordinated and strongly adopts a distorted octahedral configuration with the coordination through pyridine-N, azomethine-N, and thiolato-S atoms of the ligand. The compound crystallizes into a monoclinic lattice with the space group P21/n. The five compounds used were two monosubstituted; (i) Monomethyltin [MeSnCl₂(APCT)] abbreviated as MMT, (ii) Monophenyltin [PhSnCl₂(APCT)] abbreviated as MPT and three disubstituted organotin(IV) compounds; (iii) Dimethyltin [Me₂SnCl(APCT)] abbreviated as DMT, (iv) Dibutyltin

[Bu₂SnCl(APCT)] abbreviated as DBT and (v) Diphenyltin [Ph₂SnCl(APCT)] abbreviated as DPT.

Three levels of organotin(IV) complexes concentration of 0.1, 0.5 and 1% were prepared for treatment. The organotin(IV) complexes were dissolved in solution of 20% dimethylsulphoxide (DMSO) and 80% distilled water. The control was prepared using the same treatment solution but excluding the organotin(IV) complexes.

Ten replicates of wood cubes were used for each treatment. Treatments were carried out according to the AWP standard E10-91 with slight modifications (Rahman et al. 2013). All wood cubes were placed in beakers containing the treating solution and soaked for two hours. The beakers containing the wood cubes were then placed into a vacuum-pressure unit. The treatment schedule was done with an initial vacuum of 100 mm Hg for 30 minutes followed by 100 psi of pressure for 1 hour and a final vacuum of 100 mm Hg for 30 minutes. After treatment, the wood cubes were taken out and the excess treating solutions on the surface of the wood cubes were wiped with tissue paper and weighed (W₂).

Microorganisms, culture condition and wood biodegradation

Pure strains of *Trametes versicolor* and *Gloeophyllum trabeum* were used as the decay fungi. Both of the fungal strains were obtained from the division of Forest Products Technology, Forest Research Institute of Malaysia (FRIM). A volume of two hundred milliliters of malt extract broth (MEB) was prepared and used as the growth media for the decay fungi. Sterilization of MEB was carried out by autoclaving the media prepared at 121°C for 20 minutes.

A number of twenty mycelial blocks of each fungus were used as inoculums and cultured into malt extract broth (MEB) under static condition for two weeks at 25 °C. Grown mycelial mat were then harvested by centrifugation at 3000 rpm under 4 °C for 15 minutes and then used to inoculate the wood cubes in the wood biodegradation experiments (Liew et al. 2011).

Ten replicates for the untreated and six replicates for organotin(IV) complexes-treated wood cubes (19 mm x 19 mm x 19 mm) of *A. scholaris*, *H. brasiliensis* and *M. triloba* were used in the wood biodegradation experiments. Each beaker was loaded with one wood cube and inoculated with 10 mg of the homogenized test fungus mycelium. Cultures were maintained static at 25 °C for period of 16 weeks. Untreated wood cubes were used as the control (Liew et al. 2011).

Extraction and ligninolytic enzymes assays

After 16 weeks period of incubation, the wood cubes were subsequently subjected to a volume of 25 ml of extraction solution (50 mM sodium acetate buffer (pH 5.0) and 0.01% Tween 80) at 140 rpm (Wan & Li, 2011). Enzyme extraction was performed for 2 hrs at 20 °C ± 2 °C. The crude extract was recovered by centrifugation at 8000 rpm under 4 °C for 15 mins. Crude enzyme preparations obtained were then used in the subsequent oxidative enzyme assays.

Lignin peroxidases (LiP) activity was measured as the oxidation of veratryl alcohol (VA) to veratryl aldehyde with an increased absorbance at 310 nm. Reaction mixtures contained 50 mM sodium tartrate buffer, pH 2.5, 2.0 mM VA, 450 µl samples and 0.4 mM H₂O₂. Reaction was initiated by the

Table 1: Mean lignin peroxidase (LiP), manganese peroxidase (MnP), Laccase (Lac), weight loss (WL) and density (D) of untreated wood cubes after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Wood species	<i>Trametes versicolor</i>						<i>Gloeophyllum trabeum</i>				
	LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg/m ³)	D*** (kg/m ³)	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg/m ³)	D*** (kg/m ³)
<i>Alstonia scholaris</i>	5.39 ^a	16.23 ^a	2.72 ^a	50.52 ^a	356.27 ^a	179.69 ^a	2.88 ^a	7.88 ^a	48.40 ^a	357.82 ^a	184.15 ^a
<i>Macaranga triloba</i>	4.84 ^a	13.94 ^a	2.61 ^a	50.48 ^a	410.28 ^b	201.37 ^b	2.45 ^a	6.21 ^a	47.21 ^a	410.65 ^b	210.94 ^b
<i>Hevea brasiliensis</i>	6.57 ^b	26.72 ^b	3.17 ^b	61.66 ^b	654.99 ^c	246.85 ^c	4.21 ^b	15.47 ^b	60.13 ^b	655.93 ^c	249.87 ^c

*Means followed by a different letter within a column are statistically different at P < 0.05 using Tukey Multiple Comparison test.

**Density of wood cubes before exposure to decay fungi

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addition of H₂O₂. An extinction coefficient of 9300 M⁻¹cm⁻¹ for veratryl alcohol was used for calculation of enzyme turnover number. One unit activity was defined as the amount of enzyme oxidizing one μmol of substrate per minute (Mabrouk et al. 2010; Hariharan & Nambisan, 2013).

Manganese peroxidase (MnP) activity was determined by monitoring the oxidation of 2, 6-dimethoxyphenol (DMP) and measured as the oxidation of Mn²⁺ to Mn³⁺ by following the formation of Mn³⁺-tartrate complex at 469 nm. Reaction mixtures contained 1 mM 2,6 dimethoxyphenol (DMP), 50 mM sodium tartrate buffer, pH 4.5, 1 mM MnSO₄.H₂O, 600 μl samples and 0.4 mM H₂O₂. An extinction coefficient of 10,000 M⁻¹cm⁻¹ for Mn³⁺-tartrate complex was used for calculation of enzyme turnover number. One unit activity was defined as the amount of enzyme oxidizing one μmol of substrate per minute (Acevedo et al. 2011; Shrivastava et al. 2011).

Laccase (Lac) activity was determined using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as substrate at 420 nm. The reaction mixture contained 100 mM sodium acetate buffer, pH 5, 0.5 mM ABTS and 400 μl enzyme supernatant. An extinction coefficient of 36,000 M⁻¹cm⁻¹ for ABTS was used for calculation of enzyme turnover number. One unit activity was defined as the amount of enzyme oxidizing one μmol of substrate per minute (Mabrouk et al. 2010; Akdogan et al. 2014).

Percentage of Wood Weight Loss

The degree of fungal degradation was estimated by determining the weight loss of the wood cubes after 16 weeks period of biodegradation. The wood cubes were cleaned from the mycelium of the fungi and weighed immediately (W₄) to determine the moisture content after biodegradation. Test cubes were removed and the mycelium was wiped from the cube surfaces; the cleaned cubes were dried and conditioned at 60°C and 70% relative humidity until constant weight. The cubes were then dried in oven at 60°C until it reaches constant weight. The weights of sample cubes after drying (W₅) were recorded to determine weight loss after biodegradation.

The percentage of weight losses were calculated based on the equation 1:

$$\text{Weight loss (\%)} = \frac{W_3 - W_5}{W_3} \times 100 \quad (1)$$

Where,

W₃ = Weight of cube immediately after treatment, after conditioned at 60 °C, prior to the exposure to fungi

W₅ = Conditioned (60 °C) weight of cube after the exposure to fungal decay

Wood Density

Wood density was calculated using the ratio of weight per unit volume. The volume of the wood cubes was determined using water displacement method (Bowyer et al. 2003). The weights (W_i) and volume (V_i) of sample cubes after conditioned at 60 °C were recorded. Densities of wood cubes were calculated before and after exposed to decay fungi using equation 2 below and expressed in kg/m³.

$$\text{Density of wood (kg/m}^3\text{)} = \frac{\text{mass of wood (g)}}{\text{volume of wood (cm}^3\text{)}} \times 1000 \quad (2)$$

Data Analysis

One-way analysis of variance was used to determine the differences between mean values of lignin peroxidases, manganese peroxidases and laccases of different wood species using different concentrations of chemicals. Further analyses of mean comparisons were done employing the Tukey honest significant difference (HSD) multiple comparisons procedure (Heilmann-Clausen & Boddy, 2005; Jusoh et al. 2012). Tukey's HSD method was used because it was considered as the most appropriate for all-possible pairwise mean comparisons when sample sizes are both equal and unequal. The factors which were analyzed were concentrations of chemicals and wood species. Analysis of variance on weight loss and density of wood cubes were used to determine the mean differences among the wood species and different concentrations of chemicals using SPSS 18.0 for windows. The factors which were analyzed are chemical concentrations and wood species.

Results and Discussion

The determination of ligninolytic enzymes produced under this condition was performed to determine the actual profile of each enzyme produced during wood biodegradation. *Alstonia scholaris*, *Macaranga triloba* and *Hevea brasiliensis* wood cubes were all undergone the biodegradation by *Trametes versicolor* and *Gloeophyllum trabeum* for a period of 16 weeks. Fungal growth was found to be homogeneous throughout the biodegradation period. Ligninolytic enzymes profile of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) of the untreated wood cubes after 16 weeks of biodegradation by *T. versicolor* and *G. trabeum*, respectively were summarized (Table 1).

Wood cubes biodegraded by *T. versicolor* showed the production and detection of all ligninolytic enzymes but no Lac enzyme activities was detected when biodegraded by *G. trabeum* of all wood species. The ligninolytic enzyme activities of *H. brasiliensis* biodegraded by both *T. versicolor* and *G. trabeum* were the highest measured as compared to *A. scholaris* and *M. triloba*. The LiP, MnP

and Lac of the untreated *H. brasiliensis* wood were recorded as 6.57, 26.72 and 3.17 U/ml, respectively. The activity of laccase was the least recovered for both of the decay fungi used and in all the wood species tested.

The enzyme activity of MnP was far greater than that of LiP, suggesting that MnP might be the predominating enzymes during lignin biodegradation in the untreated tropical wood (Table 1). The higher MnP enzyme activity detected as compared to LiP and laccase might be explain due to the present of Mn-dependent peroxidase in the wood extracts if they contain enough Mn^{2+} to initiate MnP catalyzed reactions.

The highest MnP enzyme activity was previously detected from *Acacia mangium* wood chips exposed to *Trametes versicolor* among other ligninolytic enzymes (Husaini et al. 2011). LiP can also display MnP enzyme activity in the presence of H_2O_2 , O_2 , and metabolizes veratryl alcohol and oxalate, where LiP oxidizes Mn^{2+} to Mn^{3+} (Dominic, 2009; Hatakka & Hammel, 2010). The low levels of LiP enzyme activity detected compared to MnP activity showed that LiP might be of less significance in the wood lignin biodegradation by *T. versicolor* and *G. trabeum* (Dashtban et al. 2009). However, the low detectable level might be due to the present of interferences when assaying LiP using the crude enzyme extracts recovered from cultures grown on lignocellulosic materials. Typical LiP assay based on oxidation of veratryl alcohol suffers the interference of dissolved aromatic compounds present in wood extracts. (Liew et al. 2011).

The lower level of detectable laccase activity depends on the addition of easily available carbon and nitrogen sources to the culture medium (Zanirun et al 2009; Periasamy & Palvannan, 2010; Edwards et al. 2011; Mäkelä et al. 2013). This could also be due to the low content of copper (inducer of laccases) (Janusz et al 2006; Strong, 2011, Cambria et al 2011). In this study, the wood biodegradation by brown-rot fungus *G. trabeum* did not show any laccase activity (Mtui & Masalu 2008) but the white-rot fungus do produced the least amount of Lac activity during wood biodegradation. A high level of laccase enzyme activities was recorded during the biodegradation of *A. mangium* wood chips by a white-rot basidiomycete, *Pycnoporus coccineus* (Husaini et al. 2011). This might be due to the high content of copper in *A. mangium* wood (Liew et al, 2011) since it is a strong laccase inducer in the fungal species such as *T. versicolor* and *P. chrysosporium* (Dominic 2009; Dashtban et al. 2009; Dashban et al. 2010). The presence of copper did not affect fungal growth since the biomass dry weights at different times were the same in the presence and in the absence of copper (Dekker et al. 2007; Yang et al. 2013).

Trametes versicolor showed higher activities ligninolytic enzyme as compared to *Gloeophyllum trabeum* for all types of tropical wood species tested in particular *H. brasiliensis*. Brown rot fungi degrade wood using both the enzymatic and non-enzymatic processes where it selectively biodegrades the cell-wall polysaccharides, with limited lignin degradation based on both the non-enzymatic (chemical) and enzymatic attacks (Hatakka & Hammel, 2010). On the other hand, white rot fungi have wood degrading mechanisms that involved hydrolases and oxidizing enzymes and considered the only organisms capable of total mineralization of lignin (Hofrichter et al. 2010; Zeng et al. 2013). Therefore, *T. versicolor* produces more enzymes than that of *G. trabeum* during wood biodegradation process. Liew et al. (2011) recorded the highest activity of MnP than LiP and Lac from *Acacia mangium* wood chips exposed to *Trametes versicolor*. It is well established that enzyme production is highly dependent on the cultivation conditions of an organism (Yang et al. 2013). Most white rot fungi started lignin degradation when

nitrogen, carbon, or sulfur became limited (Hofrichter et al. 2010; Husaini et al. 2011).

Ligninolytic enzyme activities of LiP, MnP and Lac of the treated wood with the mono- and di-substituted organotin(IV) complexes under three level of concentrations after exposure to biodegradation by *T. versicolor* and *G. trabeum* for a period of 16 weeks were summarized in Table 2 and Table 3, respectively. Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) activities of the treated wood varied significantly with the concentration of treating organotin(IV) compounds following 16 weeks exposure to biodegradation by *T. versicolor* and *G. trabeum*. Results showed that the ligninolytic enzyme activity decreases with the increased in organotin(IV) concentrations indicating that high concentration of organotin(IV) complex inhibits the ligninolytic enzyme production. The LiP, MnP and Lac enzyme activity of monosubstituted organotin(IV)-treated wood were recorded the highest as 3.57, 15.07 and 2.02 U/ml, respectively (Table 2) whereas disubstituted organotin(IV)-treated wood were recorded the highest as 2.09, 10.41 and 1.06 U/ml, respectively (Table 3) both of *H. brasiliensis* treated wood. The use of disubstituted organotin(IV) complexes as wood preservatives significantly reduces the production of ligninolytic enzymes as compared to that of monosubstituted organotin(IV) complexes.

As a whole, the treated wood either with mono- and di-organotin(IV) produced significantly lower enzymes as compared to the untreated wood on the production of LiP, MnP and Lac. Treated wood is found to be less susceptible to enzymes involved in the degradation of wood cell or less accessible to enzymes or inhibit the fungal activity by preservatives (Elisashvili et al. 2012). Transition metal compounds of dithiocarbamates and dithiophosphinates are known antifungal agents (Kalita et al. 2002; Sonowal, 2010) and the mode of action is being the inhibitors of certain vital enzymes as the sulphur donors, the Cu^{2+} also can inhibit action of several biomolecules.

Statistical analysis were further conducted to determine the differences of lignin peroxidase, manganese peroxidase and laccase enzymes activities in relation to the chemicals effect of organotin(IV) complexes. The LiP, MnP and Lac enzyme activities of untreated and treated wood cubes with 1%-organotin(IV) complexes after 16 weeks exposure to *T. versicolor* and *G. trabeum* among wood species were evaluated (Fig. 1 – Fig. 5).

The untreated woods ligninolytic enzyme activities are significantly higher than that of treated woods of both biodegraded by *T. versicolor* and *G. trabeum*. Among the monosubstituted organotin(IV)-treated wood, MMT showed higher ligninolytic enzyme activities than MPT. As for the disubstituted organotin(IV)-treated woods, the lowest and highest ligninolytic enzyme activity were determined of DBT and DMT, respectively.

Lignin peroxidase activity by *T. versicolor* (Fig. 1) was higher than that of *G. trabeum* (Fig. 4) on all wood species tested. The activities of LiP in untreated wood is significantly higher than that of treated wood samples with organotin(IV) complexes. Manganese peroxidase activity was almost two fold higher than that of lignin peroxidase (Fig. 1, Fig. 2, Fig. 4 and Fig. 5). Laccase activities of untreated wood by *T. versicolor* were significantly higher than treated wood (Fig. 3). *G. trabeum* did not show any laccase activity. DBT-treated wood sample

Table 2: Mean lignin peroxidase (LiP), manganese peroxidase (MnP), Laccase (Lac), weight loss (WL) and density (D) of treated wood cubes with monosubstituted organotin(IV) complexes after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Treating chemicals	Wood species	Concentration (%)	<i>Trametes versicolor</i>					<i>Gloeophyllum trabeum</i>					
			LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg m ⁻³)	D*** (kg m ⁻³)	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg m ⁻³)	D*** (kg m ⁻³)
MMT	<i>Alstonia scholaris</i>	0.1	2.06 ^{ab}	7.99 ^b	1.01 ^b	22.31 ^c	357.66 ^a	260.61 ^a	1.91 ^b	5.09 ^c	25.05 ^c	359.22 ^a	254.61 ^a
		0.5	1.79 ^{ab}	7.53 ^b	0.89 ^{ab}	18.24 ^b	359.02 ^a	279.51 ^b	1.71 ^{ab}	4.01 ^b	20.11 ^b	360.61 ^a	270.73 ^b
		1	1.51 ^a	6.04 ^a	0.81 ^a	14.38 ^a	361.52 ^a	291.90 ^c	1.49 ^a	3.02 ^a	16.12 ^a	362.40 ^a	291.24 ^c
	<i>Macaranga triloba</i>	0.1	2.01 ^b	7.01 ^b	1.01 ^b	25.63 ^c	409.53 ^a	310.37 ^a	1.91 ^b	5.09 ^c	25.78 ^b	410.05 ^a	304.44 ^a
		0.5	1.71 ^{ab}	6.53 ^{ab}	0.85 ^{ab}	19.92 ^b	410.16 ^a	318.45 ^{ab}	1.60 ^a	3.99 ^b	20.61 ^{ab}	413.28 ^a	314.34 ^a
		1	1.40 ^a	5.88 ^a	0.74 ^a	15.48 ^a	413.68 ^a	334.07 ^b	1.32 ^a	3.01 ^a	17.56 ^a	414.7 ^a	331.41 ^b
	<i>Hevea brasiliensis</i>	0.1	3.57 ^c	15.07 ^d	2.02 ^c	23.31 ^b	656.03 ^b	509.81 ^a	2.52 ^c	7.44 ^d	24.13 ^c	657.41 ^b	502.88 ^b
		0.5	3.06 ^b	13.33 ^b	1.51 ^b	18.43 ^b	658.22 ^a	530.01 ^b	2.02 ^b	6.04 ^c	19.94 ^b	659.39 ^a	525.51 ^b
		1	2.51 ^a	10.23 ^a	1.10 ^a	13.01 ^a	661.88 ^a	551.14 ^c	1.48 ^a	4.99 ^a	14.62 ^a	661.21 ^a	541.15 ^c
MPT	<i>Alstonia scholaris</i>	0.1	1.92 ^a	7.01 ^b	0.99 ^a	19.83 ^b	358.27 ^a	265.21 ^b	1.84 ^b	4.05 ^c	22.88 ^c	358.72 ^a	259.11 ^a
		0.5	1.69 ^{ab}	6.11 ^{ab}	0.89 ^{ab}	14.58 ^b	359.22 ^a	287.12 ^b	1.65 ^{ab}	3.07 ^b	16.61 ^b	359.44 ^a	274.84 ^b
		1	1.32 ^a	5.54 ^a	0.79 ^a	10.35 ^a	361.81 ^a	298.07 ^c	1.30 ^a	2.08 ^a	10.13 ^a	361.06 ^a	296.24 ^c
	<i>Macaranga triloba</i>	0.1	1.82 ^a	6.13 ^b	0.91 ^a	23.81 ^c	409.66 ^a	314.04 ^a	1.02 ^a	3.18 ^b	24.78 ^b	411.72 ^a	309.94 ^a
		0.5	1.51 ^{ab}	5.08 ^a	0.85 ^a	16.75 ^b	412.99 ^a	325.95 ^b	0.83 ^a	2.53 ^{ab}	16.44 ^b	413.78 ^a	319.18 ^{ab}
		1	1.29 ^a	4.26 ^a	0.62 ^a	10.68 ^a	416.34 ^a	340.53 ^c	0.63 ^a	1.91 ^a	9.96 ^a	415.90 ^a	329.91 ^b
	<i>Hevea brasiliensis</i>	0.1	3.07 ^b	14.41 ^d	1.44 ^b	21.14 ^c	655.86 ^b	521.47 ^b	2.08 ^b	6.44 ^d	23.96 ^c	656.08 ^b	519.22 ^b
		0.5	2.51 ^{ab}	12.51 ^{cd}	1.29 ^b	16.09 ^b	657.55 ^b	544.35 ^b	1.53 ^a	5.09 ^c	17.44 ^b	658.59 ^b	537.85 ^b
		1	2.09	9.73 ^a	0.91 ^a	10.35 ^a	659.88 ^a	560.81 ^c	1.02 ^a	4.19 ^a	11.66 ^a	660.45 ^a	555.99 ^c

MMT- Monomethyltin(IV) complex, MPT- Monophenyltin(IV) complex

*Means followed by a different letter within a column are statistically different at P < 0.05 using Tukey Multiple Comparison test.

**Density of wood cubes before exposure to decay fungi

***Density of wood cubes after exposure to decay fungi

Table 3: Mean lignin peroxidase (LiP), manganese peroxidase (MnP), laccase (Lac), weight loss (WL) and density (D) of treated wood cubes with disubstituted organotin(IV) complexes after 16 weeks exposure to *T. versicolor* and *G. trabeum*.

Treating chemicals	Wood species	Concentration (%)	<i>Trametes versicolor</i>					<i>Gloeophyllum trabeum</i>					
			LiP (U/ml)	MnP (U/ml)	Lac (U/ml)	WL (%)	D** (kg m ⁻³)	D*** (kg m ⁻³)	LiP (U/ml)	MnP (U/ml)	WL (%)	D** (kg m ⁻³)	D*** (kg m ⁻³)
DMT	<i>Alstonia scholaris</i>	0.1	1.10 ^{ab}	5.96 ^c	0.84 ^a	16.83 ^c	357.16 ^a	276.21 ^a	0.93 ^b	3.02 ^b	19.38 ^c	359.72 ^a	268.27 ^a
		0.5	0.85 ^{ab}	4.07 ^a	0.67 ^a	11.92 ^b	359.88 ^a	294.62 ^b	0.82 ^{ab}	2.56 ^{ab}	12.56 ^b	362.06 ^a	291.34 ^b
		1	0.68 ^a	2.94 ^a	0.46 ^a	9.18 ^a	362.08 ^a	318.07 ^c	0.60 ^a	2.08 ^a	9.46 ^a	364.44 ^a	312.57 ^c
	<i>Macaranga triloba</i>	0.1	1.58 ^b	6.03 ^b	0.79 ^a	17.63 ^c	409.83 ^a	336.37 ^a	0.91 ^b	3.06 ^b	19.11 ^c	409.59 ^a	331.44 ^a
		0.5	1.24 ^{ab}	4.94 ^{ab}	0.58 ^a	14.42 ^b	411.34 ^a	344.12 ^b	0.75 ^{ab}	2.43 ^{ab}	13.94 ^b	410.40 ^a	338.18 ^{ab}
		1	1.05 ^a	4.16 ^a	0.51 ^a	8.21 ^a	414.66 ^a	358.91 ^c	0.49 ^a	1.51 ^a	8.29 ^a	413.44 ^a	356.07 ^b
	<i>Hevea brasiliensis</i>	0.1	2.09 ^b	10.41 ^d	1.06 ^b	20.48 ^c	657.23 ^b	548.3 ^a	1.03 ^b	3.99 ^b	21.46 ^c	659.75 ^b	539.05 ^a
		0.5	1.45 ^{ab}	6.90 ^a	0.87 ^a	13.46 ^b	660.92 ^a	576.68 ^b	0.85 ^{ab}	2.40 ^b	15.27 ^b	661.92 ^b	570.68 ^{ab}
		1	1.06 ^a	6.56 ^a	0.59 ^a	9.06 ^a	664.51 ^a	596.97 ^c	0.53 ^a	2.34 ^a	9.96 ^a	664.62 ^a	583.32 ^b
DPT	<i>Alstonia scholaris</i>	0.1	0.77 ^a	3.04 ^a	0.29 ^a	16.33 ^c	357.36 ^a	286.21 ^a	0.54 ^a	1.90 ^b	18.05 ^c	357.05 ^a	281.11 ^a
		0.5	0.48 ^{ab}	1.94 ^a	0.18 ^{ab}	10.92 ^b	361.24 ^a	311.62 ^b	0.36 ^{ab}	1.43 ^{ab}	13.51 ^b	361.11 ^a	301.34 ^a
		1	0.28 ^a	0.96 ^a	0.08 ^a	7.18 ^a	364.38 ^a	334.24 ^c	0.18 ^a	0.89 ^a	8.26 ^a	363.73 ^a	329.24 ^b
	<i>Macaranga triloba</i>	0.1	1.24 ^b	4.03 ^b	0.54 ^b	17.31 ^c	411.01 ^a	337.37 ^a	0.67 ^b	1.99 ^b	18.78 ^c	410.22 ^a	326.77 ^a
		0.5	0.99 ^{ab}	3.02 ^b	0.43 ^{ab}	11.42 ^b	412.99 ^a	357.45 ^{ab}	0.48 ^{ab}	1.53 ^{ab}	13.49 ^b	412.11 ^a	352.01 ^b
		1	0.78 ^a	1.92 ^a	0.18 ^a	6.15 ^a	415.51 ^a	374.57 ^b	0.31 ^a	1.01 ^a	7.49 ^a	414.23 ^a	368.24 ^b
	<i>Hevea brasiliensis</i>	0.1	1.46 ^b	6.41 ^b	0.81 ^b	18.14 ^c	661.56 ^a	580.97 ^a	0.76 ^b	3.09 ^b	18.46 ^c	659.10 ^a	577.22 ^a
		0.5	1.25 ^{ab}	5.00 ^{ab}	0.59 ^{ab}	9.26 ^b	662.59 ^a	588.68 ^{ab}	0.52 ^{ab}	2.31 ^{ab}	10.57 ^b	663.59 ^a	582.51 ^{ab}
		1	0.92 ^a	4.19 ^a	0.39 ^a	7.02 ^a	664.51 ^a	601.97 ^b	0.34 ^a	2.09 ^a	7.45 ^a	666.45 ^a	595.99 ^b
DBT	<i>Alstonia scholaris</i>	0.1	0.54 ^a	1.97 ^a	0.24 ^a	13.49 ^c	360.52 ^a	332.87 ^a	0.42 ^a	1.51 ^b	15.55 ^c	360.39 ^a	328.27 ^a
		0.5	0.33 ^{ab}	1.43 ^{ab}	0.13 ^a	9.42 ^b	361.41 ^a	345.79 ^{ab}	0.22 ^{ab}	1.30 ^{ab}	10.27 ^b	362.78 ^a	341.18 ^{ab}
		1	0.16 ^a	0.93 ^a	0.06 ^a	3.28 ^a	363.21 ^a	353.24 ^b	0.11 ^a	0.81 ^a	4.28 ^a	365.40 ^a	347.40 ^b
	<i>Macaranga triloba</i>	0.1	0.86 ^a	2.11 ^b	0.24 ^a	15.97 ^c	410.66 ^a	388.54 ^a	0.43 ^b	1.13 ^b	15.95 ^c	412.39 ^a	385.11 ^a
		0.5	0.63 ^{ab}	1.49 ^{ab}	0.15 ^a	8.42 ^b	412.49 ^a	397.12 ^{ab}	0.24 ^{ab}	0.92 ^{ab}	9.44 ^b	413.78 ^a	393.18 ^{ab}
		1	0.35 ^a	1.03 ^a	0.08 ^a	4.15 ^a	414.34 ^a	404.39 ^b	0.12 ^a	0.60 ^a	4.71 ^a	415.06 ^a	402.07 ^b
	<i>Hevea brasiliensis</i>	0.1	0.72 ^a	2.08 ^b	0.21 ^a	14.81 ^c	661.40 ^a	593.31 ^a	0.44 ^b	1.08 ^b	14.80 ^c	660.76 ^a	585.55 ^a
		0.5	0.51 ^{ab}	1.50 ^{ab}	0.15 ^a	7.43 ^b	663.59 ^a	599.01 ^a	0.31 ^{ab}	0.82 ^{ab}	8.44 ^b	663.26 ^a	596.18 ^a
		1	0.34 ^a	1.13 ^a	0.07 ^a	3.55 ^a	664.51 ^a	560.81 ^c	0.13 ^a	0.61 ^a	4.18 ^a	666.62 ^a	615.49 ^b

DMT- Dimethyltin(IV) complex, DPT- Diphenyltin(IV) complex, DBT- Dibutyltin(IV) complex

*Means followed by a different letter within a column are statistically different at P < 0.05 using Tukey Multiple Comparison test.

Density of wood cubes before exposure to decay fungi; *Density of wood cubes after exposure to decay fungi

significantly reduces the production of LiP, MnP and Lac activities than that of other organotin(IV) complexes in all wood species.

The weight loss and density of the untreated and organotin(IV)-treated wood cubes were conducted to relate the production of the ligninolytic enzyme production and the wood biodegradation in particular the lignin degradation. The weight loss and density are good indicators on assessing the wood biodegradation as a whole.

In general, the percentages of weight losses due to *T. versicolor* were found significantly higher than those of *G. trabeum* after 16 weeks of wood biodegradation across all wood species. The percentage of weight loss of the untreated *H. brasiliensis* wood cubes was recorded the highest of 62% and 60% after exposure to

wood biodegradation by *T. versicolor* and *G. trabeum*, respectively (Table 1). Interestingly, the increased percentage of weight loss was in parallel with the production of ligninolytic enzymes in all of the wood species and the highest was recorded for untreated *H. brasiliensis* wood cubes as compared to *M. triloba* and *A. scholaris*.

The presence of carbohydrate in *H. brasiliensis* is high which makes it highly susceptible to decay (Florence et al. 2002; Rahman et al. 2013). The variation in terms of the percentage of weight loss might be due to important physical decay experimental parameters such as moisture content, temperature and pH. Moreover, other intrinsic factors such as decay rate of

the fungi, genetic factors, adaptability, wood species, and survivability of fungi are also important.

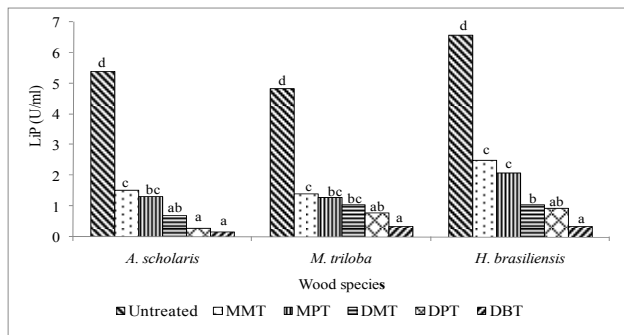


Figure 1: Lignin peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

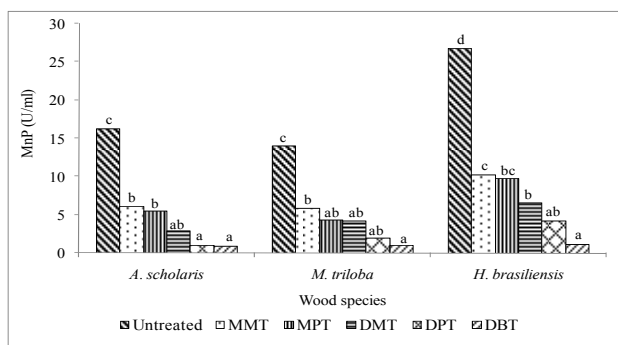


Figure 2: Manganese peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

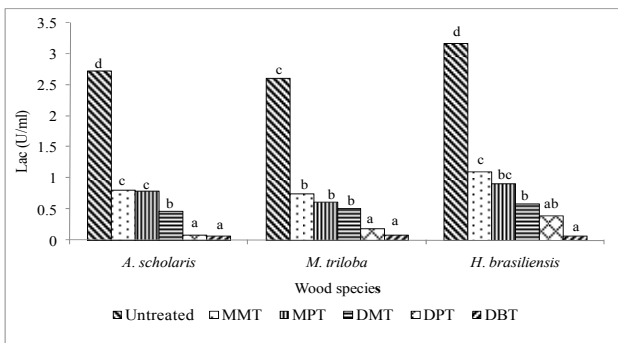


Figure 3: Laccase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Trametes versicolor*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

Wood density is an indicator of wood quality (Bowyer et al. 2003; Chave et al. 2009). As for the untreated woods before and after the exposure to the biodegradation of fungi, the mean wood densities were found to be significantly different among the wood species (Table 1). Wood densities of the untreated *A. scholaris*, *M. triloba* and *H. brasiliensis* were initially recorded as 357, 410 and 655 kg/m^3 , respectively. After 16 weeks of exposure to wood biodegradation by *T. versicolor* and *G. trabeum* the wood densities were reduced and recorded varied from 180 to 184, 201 to 211 and 247 to 250 kg/m^3 , respectively. The reductions of wood densities are

in parallel with the increased in the production of ligninolytic enzymes produced. The reduction was found to be parallel with the percentage weight loss of all the wood species. Wood density variation may occur within a species due to the location within the tree, site condition, genetic factor and age of tree (Chave et al. 2009).

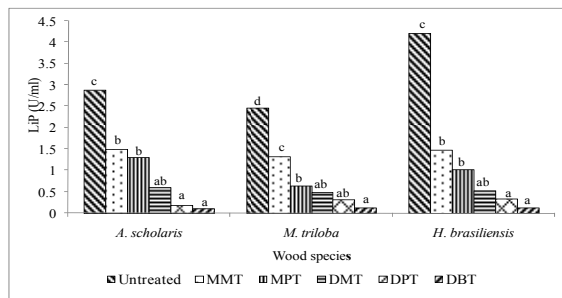


Figure 4: Lignin peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Gloeophyllum trabeum*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

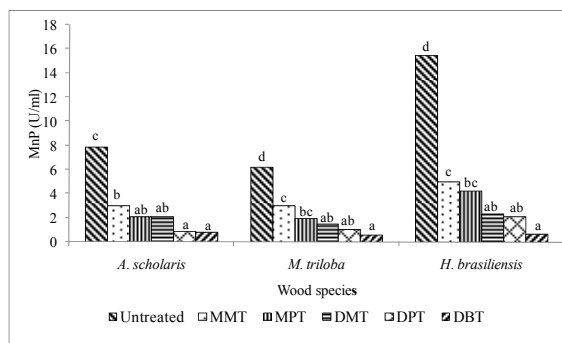


Figure 5: Manganese peroxidase (U/ml) activities of untreated and treated wood cubes with (1%) organotin(IV) complex after 16 weeks exposure to *Gloeophyllum trabeum*. Different letter within wood species are statistically different at $P < 0.05$ using Tukey Multiple Comparison test.

Interestingly, the densities of the treated wood after the exposure to *T. versicolor* and *G. trabeum* biodegradation increased significantly among all of the wood species in correlation with the increase in the chemical concentrations used. The mean densities of the treated *A. scholaris*, *M. triloba* and *H. brasiliensis* were initially recorded the highest as 365, 416 and 667 kg/m^3 , respectively. After 16 weeks of exposure to fungal biodegradation, the mean densities of the treated *A. scholaris*, *M. triloba* and *H. brasiliensis* slightly reduced to the maximum of 353, 404 and 622 kg/m^3 , respectively. The least reduction in the mean densities indicates lesser degree of wood degradation of the organotin(IV) complexes-treated wood in comparison to the untreated wood. The minimal reduction of the treated wood densities indicates that organotin(IV) complexes significantly protect the wood biomass from the fungal degradation through the reduced and least amount of ligninolytic enzymes produced compared to untreated wood.

Conclusions

In this study, it has been successfully indicated that based on the ligninolytic enzyme analysis, percentage of weight loss and wood densities revealed that dibutyltin(IV) complex treated wood is the best protection as wood preservative against

Trametes versicolor and *Gloeophyllum trabeum*. Ligninolytic enzymes profile of the untreated non-durable wood species biodegraded, highlighted the production of the main enzyme, manganese peroxidase as the predominant enzyme expressed during wood biodegradation. The least amount of laccase activity was detected only from *T. versicolor* and as well as producing the most level of ligninolytic enzymes detected. It can be concluded that the newly synthesized organotin(IV) complexes are effective in reducing the production of ligninolytic enzymes during wood biodegradation that signify it as wood preservatives where DBT complex is determined as the best wood preservative among all the tested organotin(IV) complexes.

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References

- Affan MA, Salam MA, Ahmad FB, Ismail J, Shamsuddin MB, Ali HM (2011) Synthesis and spectroscopic characterization of organotin(IV) complexes with 2-benzoylpyridine-N(4)-cyclohexylthiosemicarbazone (HBPCT): X-ray crystal structure of [PhSnCl₂(BPCT)]. *Inorg Chim Acta* 366: 227-232
- Acevedo F, Pizzul L, Castillo MP, Rubilar O, Lienqueo ME, Tortella G, Diez MC (2011) A practical culture technique for enhanced production of manganese peroxidase by *Anthracoephyllum discolor* Sp4 Braz. Arch. Biol. Technol. 54 (6): 1175-1186.
- Akdogan HA, Merve C Topuz MC, Urhan AA, (2014) Studies on decolorization of reactiveblue 19 textile dye by *Coprinus plicatilis*. *Journal of Environmental Health Science & Engineering* 12:49.
- Asgher M, Sharif Y, Bhatti HN (2010) Enhanced production of ligninolytic enzymes by *Ganoderma lucidum* IBL-06 using lignocellulosic agricultural wastes. *Int J Chem React Eng* 8(1): 1-19
- Bowyer JL, Schmulsky R, Haygreen JG (2003) *Forest products and wood science: An introduction*. 4th edn. Iowa State Press, Iowa
- Cambria MT, Ragusa S, Calabrese V, Cambria A (2011) Enhanced laccase production in white-rot fungus *Rigidoporus lignosus* by the addition of selected phenolic and aromatic compounds. *Appl Biochem Biotechnol* 163: 415-422.
- Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE (2009) Towards a worldwide wood economics spectrum. *Ecol Lett* 12:351-366
- Dashtban M, Schraft H, Qin W, (2009) Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. *Int J Biol Sci.* 5:578-595
- Dashtban M, Schraft H, Syed TA, Qin W (2010) Fungal biodegradation and enzymatic modification of lignin *Int J Biochem Mol Biol* 1(1):36-50
- Dekker RFH, Barbosa AM, Giese EC, Godoy SDS, Covizzi LG (2007) Influence of nutrients on enhancing laccase production by *Botryosphaeria rhodina* MAMB-05 *Int Microbiol* 10(3):177-185
- Dominic W. S. Wong (2009) Structure and action mechanism of ligninolytic enzymes. *Appl Biochem Biotechnol* 157:174-209
- Edwards IP, Zak DR, Kellner H, Eisenlord SD, Pregitzer KS. (2011) Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a northern hardwood forest. *PLoS One.* 6:1-10.
- Elisashvili V, Irbe I, Andersone I, Andersons B, Tsiklauri N (2012) Hydrolytic enzyme activity of EN113 standard basidiomycetes in the fermentation of lignocellulosic material and wood colonization. *Holzforschung* 66: 841-847
- Hamed SAM (2013), In-vitro studies on wood degradation in soil by soft-rot fungi: *Aspergillus niger* and *Penicillium chrysogenum* *International Biodeterioration and Biodegradation* 78: 98-102.
- Hariharan S, Nambisan P (2013) Optimization of lignin peroxidase, manganese peroxidase, and Lac production from *Ganoderma lucidum* under solid state fermentation of pineapple leaf. *Bioresources* 8(1):250-271
- Hatakka A, Hammel KE (2010) Fungal biodegradation of lignocelluloses: In *Industrial Applications*, 2nd Edition The Mycota X Hofrichter M. (Ed.) Springer-Verlag Berlin Heidelberg
- Heilmann-Clausen J., Boddy L. (2005) Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species *Microbial ecology*, 49: 399-406.
- Hofrichter M, Ullrich R, Pecyna MJ, Liers C, Lundell T (2010) New and classic families of secreted fungal heme peroxidases. *Appl Microbiol Biotechnol* 87:871-897
- Husaini A, Fisol FA, Yun LC, Hussain MH, Muid S, Roslan HA (2011) Lignocellulolytic enzymes produced by tropical white rot fungi during biopulping of *Acacia mangium* wood chips. *J Biochem Tech* 3(2): 245-250
- Iqbal HMN, Asgher M, Bhatti HN (2011) Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by novel strain of *Trametes versicolor*. *BioResources* 6(2): 1273-1287
- Janusz G, Rogalski J, Barwinska M, Szczodrak J, (2006) Effects of culture conditions on production of extracellular laccase by *Rhizoctonia praticola* *Polish Journal of Microbiology*, 55 (4): 309-319
- Jusoh I, Rahman MM, Affan MA, Hamdan S, Salam MA (2012) Treatability of tropical wood using newly synthesized organotin(IV) complexes. *J Applied Sci* 12(6):535-543
- Juthika Sonowal (2010) Dimensional stability, thermal degradation and termite resistant studies of chemically treated Wood *International Journal of Chemistry* 2(2): 218-225
- Kalita DK, Das DK, Gogoi PK (2002) Synthesis, characterization and antibacterial activity of 2-methylpiperazine dithiocarbamates of Ru(III), Rh(III), Pd(II), Os(III) and Pt(II). *Proceedings of National Academy of Sciences, India*, 72(A): 7-13
- Liew CY, Husaini A, Hussain H, Muid S, Liew KC, Roslan HA (2011) Lignin biodegradation and ligninolytic enzyme studies during biopulping of *Acacia mangium* wood chips by tropical white rot fungi. *World J Microbiol Biotechnol* 27(6): 1457-1468
- Mabrouk AM, Kheiralla ZH, Hamed ER, Youssry AA, Abeer, Abd El Aty A. (2010) Screening of some marine-derived fungal isolates for lignin degrading enzymes (LDEs) production *Agric. Biol. J. N. Am.*, 1(4): 591-599
- Mäkelä MR, Lundell T, Hatakka A, Hildén K. (2013) Effect of copper, nutrient nitrogen, and wood-supplement on the production of lignin-modifying enzymes by the white-rot fungus *Phlebia radiata*. *Fungal Biol.* 117(1):62-70

- Mtui G, Masalu R (2008) Extracellular enzymes from brown-rot fungus *Laetiporus sulphureus* isolated from mangrove forests of coastal Tanzania. *Sci Res Essays* 3(4): 154-161
- Patrick F, Mtui G, Mshandete AM, Kivaisi A (2011) Optimization of laccase and manganese peroxidase production in submerged culture of *Pleurotus sajor-caju*. *Afr J Biotechnol* 10(50): 10166-10177
- Rahman MM, Jusoh I, Affan MA, Husaini A, Hamdan S (2013) Efficacy of novel organotin(IV) complexes on non-durable tropical wood against decay fungi. *Eur. J. Wood Prod.* (2013) 71:463-471
- Salam MA, Affan MA, Arafat MA, Saha R, Nasrin R (2013) Synthesis, characterization, and antibacterial activities of organotin(IV) complexes with 2-acetylpyridine-N(4)-cyclohexylthiosemicarbazone (HAPCT). *Heteroatom Chem* 24(1): 43-52
- Sasaki T, Kajino T, Li B, Sugiyama H, Takahashi H (2001) New pulp biobleaching system involving manganese peroxidase immobilized in a silica support with controlled pore sizes. *Appl Environ Microbiol* 67(50): 2208-2212
- Shrivastava, B., Thakur, S., Khasa, Y. P., Gupte, A., Puniya, A. K., & Kuhad, R. C. (2011). White-rot fungal conversion of wheat straw to energy rich cattle feed. *Biodegradation*, 22(4), 823-831.
- Skyba, O., Douglas, C. J., Mansfield, S. D. (2013) Syringyl-rich lignin renders poplars more resistant to degradation by wood decay fungi. *Applied and environmental microbiology*, 79(8), 2560-2571.
- Strong PJ, (2011) Improved Laccase Production by *Trametes pubescens* MB89 in Distillery Wastewaters,” *Enzyme Research*, vol. 2011, Article ID 379176, 8 pages, 2011. doi:10.4061/2011/379176
- Tanka, H., Koike, K., Itakura, S., Enoki, A., (2009). Degradation of wood and enzyme production by *Ceriporiopsis subvermispora*. *Enzyme and Microbial Technology* 45: 384-390
- Wan, C., & Li, Y. (2011). Effectiveness of microbial pretreatment by *Ceriporiopsis subvermispora* on different biomass feedstocks. *Bioresour. Technol.*, 102(16): 7507-7512.
- Yang Y, Wei F, Zhuo R, Fan F, Liu H, et al. (2013) Enhancing the laccase production and laccase gene expression in the white-rot fungus *Trametes velutina* 5930 with great potential for biotechnological applications by different metal ions and aromatic compounds. *PLoS ONE* 8(11): e79307. doi:10.1371/journal.pone.0079307
- Zanirun Z, Abd-Aziz S, Ling FH, Hassan MA, (2009). Optimisation of lignin peroxidase production using locally isolated *Pycnoporus* sp. through factorial design. *Biotechnology*, 8: 296-305.
- Zeng GM, Zhao MH, Huang DL, Lai C, Huang C, Wei Z, Xu P, Li NJ, Zhang C, Li FL, Cheng M (2013). Purification and biochemical characterization of two extracellular peroxidases from *Phanerochaete chrysosporium* responsible for lignin biodegradation. *International Biodeterioration & Biodegradation* 85:166-17