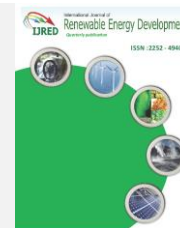Contents list available at IJRED website

**Int. Journal of Renewable Energy Development (IJRED)**

Journal homepage: http://ejournal.undip.ac.id/index.php/ijred

# The Characteristic Changes of Betung Bamboo (*Dendrocalamus asper*) Pretreated by Fungal Pretreatment

Widya Fatriasari[a,b,*], Wasrin Syafii[b], Nyoman J Wistara[b], Khaswar Syamsu[c] and Bambang Prasetya[d]

[a] *Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), INDONESIA*

[b] *Department of Forest Product Technology, Faculty of Forestry, Bogor Agricultural University, INDONESIA*

[c] *Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, INDONESIA*

[d] *National Standardization Agency, INDONESIA*

**ABSTRACT**: The fungal pretreatment effect on chemical structural and morphological changes of betung bamboo was evaluated based on its biomass components after being cultivated by white rot fungi, *Trametes versicolor*. Betung bamboo powder (15 g) was exposed to liquid inoculum of white rot fungi and incubated at $27^0$C for 15, 30 and 45 days. The treated samples were then characterized by FT-IR spectroscopy, X-Ray diffraction and SEM-EDS analyses. Cultivation for 30 days with 5 and 10% loadings retained greater selectivity compared to that of the other treatments. FTIR spectra demonstrated that the fungus changed the chemical structure of pretreated bambooo indicated by the decreasing of functional group intensities without changing the functional groups. The decrease in intensity at wave number of 1246 $cm^{-1}$ (guaiacyl of lignin) was greater than that at wave number of 1328 $cm^{-1}$ (deformation combination of syringyl and xylan) after fungal treatment. X-ray analysis showed the pretreated samples had a higher crystallinity than the untreated ones which might be due to the cleavage of amorphous fractions of cellulose. The pretreated bamboo have more fragile than the untreated ones confirmed by SEM. Crystalline allomorph, $I_\beta$ (monoclinic) has transformed $I\alpha$ (triclinic) structure in the pretreated bamboo for 30 days.

## 1. Introduction

Depleting stock of fossil fuel has promoted worldwide research and development of bioethanol for fossil fuels substitute. Lignocellulosic biomass is the most promising raw material for ethanol production considering its abundance and distribution. However, crystalline nature and the presence of lignin in lignocellulosic biomass hinder enzyme penetration during hydrolysis process resulting in low cellulose conversion (Mosier *et al*. 2005). To allow the biological agents to effectively hydrolyze carbohydrate, the pretreatment step is required to alter the recalcitrance structure of biomass to increase the surface area, porosity, reducing cellulose crystallinity and lignin content, and removing hemicellulose as well (Gelbe & Zacchi 2007, Wyman 2007, Cara *et al*. 2008). The effective pretreatment can be recognized by the improvement of sugar release and at the same time avoid carbohydrate degradation and inhibitory by-products formation such as furfural, hydroxymethylfurfural (HMF), and several organic acids (Larsson *et al*. 1999) for subsequent process and also be cost-effective (Sun & Cheng 2002).

---

* Corresponding author: +62-021-87914511
Email: widya_fatriasari@yahoo.com

The increasing environment-friendly concern leads to the development of pretreatment approach which has low energy input, mild environmental conditions, no chemical consumption and simple procedure requirement and equipment (Sun & Cheng 2002). Biological pretreatment is a promising technique which utilizes microorganisms to remove lignin polymer efficiently from plant cell (Hakala *et al.* 2005, Akhtar *et al.*1998, Eriksson *et al.*1990). White-rot fungi are the most effective basidiomycetes for this pretreatment of lignocellulosic materials (Sun & Cheng 2002, Hakala *et al.* 2005, Akhtar *et al.* 1998, Eriksson *et al.* 1990, Zhang *et al.* 2007). These fungi secrete lignolysis degrading enzymes i.e lignin peroxidase (LiP), laccase, mangan peroxidase (MnP) and versatile peroxidase to penetrate into the plant tissues which further perform the degrading action on the lignocellulosic substrate (Messner & Srebotnik 1994, Kirk & Chang 1981). Biological degradation process of lignocelluloses is a complex process in which the fungi strain, culture condition, oxidative mechanism and fungi enzymatic secretion affect the obtained result (Guillen *et al.* 2000, Wan & Li 2010). These factors could affect delignification selectivity which will lead to obtain higher sugar yield from enzymatic hydrolysis if this selectivity value is greater than two (Giles *et al.* 2011). The fungi strain selection with appropriate substrate condition is required. The previous research reported that *Trametes versicolor* applied on betung bamboo (*Dendrocalamus asper*) showed better delignification selectivity than that of *Pleurotus ostreatus* and *Phanerochaete chrysosporium* (Fatriasari *et al.* 2011, Falah *et al.* 2011).

Bamboos typified as plants belonging to the grasses family afford very efficient photosynthesis ability as shown by the high production of this lignocellulosic biomass (20-40 tonnes/ha/year) and this production is about 7-30% higher than that of woody plants (Kant 2010). Moreover, compared to other energy crops (poplar, switch grass, miscanthus, common reed, and bagasse (Shathitsuksanoh *et al.* 2009, Zhang 2008), the biomass production of bamboos is superior. These plants are known potential source of fiber which have wide area utilization such as pulp and paper production, lactid acid, composite reinforcement, methane, construction, textile, food and bioenergy. Bamboo plants cover 37 million hectares (1% of the world's forest area) (Kant 2010) and 65% of the population is in Asia. Indonesia's bamboo production ranks third (5%), which consists of 160 species (Widjaja *et al.* 2011) after India (30%) and China (14%) (Lobovikov *et al.* 2007). Bamboos are easily and quickly produced (Scurlock *et al.* 2000, Gratani *et al.* 2008), with the production of cellulose/ha 2-6 times higher than pine (Nasendi 1995). Betung bamboo is one of the most important bamboo species in Indonesia (Dransfield & Widjaja 1995) and this bamboo has fiber morphology and physical-chemical properties which are better than those of other bamboos such as andong, ampel and black bamboos (Fatriasari & Hermiati 2008).

Detail study of carbohydrate structure after pretreatment of non woody biomass such as bamboo originated from Indonesia is less reported in previous studies. Therefore, this research focused on the changes of cellulose structure and lignin content after biological pretreatment of betung bamboo using white rot fungi, *T.versicolor*. The effect of incubation time and inoculum loading was investigated in relation to its possibility to affect hydrolysis performance.

## 2. Materials and Methods

### 2.1 Material Preparation

Fresh and barkless 2 year-old Bamboo Betung (*Dendrocalamus asper* (Schult.f.)), originated from bamboo garden of Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Cibinong Indonesia was cut using a drum chipper, ring flaker, hammermil and disk mill to meet bamboo powder in size of 40-60 mesh.

### 2.2 Biological Pretreatment

Bamboo powder of 40-60 mesh was watered with a ratio of 1:4 and then stirred until completely mixed. The wet bamboo powder was then put in a jar and steamed for 30 minutes at temperature of ± 100 °C and finally autoclaved for 20 minutes at a temperature of $121^0$C and a pressure of 1 atm.

Microorganism cultivar were prepared by inoculating *T.versicolor* on malt extract agar (MEA) slant (10.65 grams of MEA were diluted in 300 ml destillated water) for 7-14 days. At the end of incubation period, 5 ml JIS (Japan Industrial Standard) broth medium (3 g $KH_2PO_4$, 2 g $MgSO_4.7H_2O$, 25 g glucose, 5 g peptone, and 5 g malt extract in 1 L destillated water) was injected to slant and then scratched by loop to release the resulting mycelium from the agar. As much as 5 ml of the previously prepared fungi suspension was then poured into the 95 ml of the JIS Broth medium in 300 ml elemeter flask and incubated stationery at $27^0$C for 7-10 days. After incubation, 10 grams of corn steep liquor was then poured into the 100 ml inoculum. They were then homogenized in high speed waring blender for 40 seconds.

The pretreatment was conducted by inoculating the culture of *T.versicolor* (5 and 10% w/v of dry bamboo) into medium containing bamboo powder, and were incubated at 27 °C up to 15, 30 and 45 days.

### 2.3 The changes of cellulose and lignin characteristics

### 2.3.1 Chemical component measurement

Determination of chemical component of bamboo was measured before and after pretreatment to compare the changes of the properties. Moisture content of bamboo was measured prior to chemical component determination based on TAPPI T12 os-75. Firstly, bamboo was extracted by ethanol-benzene (1:2) prior to

determination of acid-insoluble lignin content (TAPPI T13 os-54), holocellulose (TAPPI T9m-54), and alpha cellulose (Mokushitsu Kagaku Jiken Manual 2000) and ash content (T 15 os-58).

### 2.3.2 Determination of cellulose crystallinity index

The X-ray Diffraction (XRD) analysis was used to calculate the cellulose crystallinity index according to the formulation of Zhao *et al.* (2006) and Foscher *et al.* (2001), based on diffraction intensity data. Dried bamboo of 40-60 mesh was placed in holder glass and analyzed in room temperature and recorded with Shimadzu XRD-700 MaximaX series. NI radiation was filtered by $CuK_\alpha$ at 0.15406 nm wave number. X-ray was operated at 40 kV of voltage, 30 mA of electrical current and scanned 2θ angles of 10-40° in 2°/minutes.

### 2.3.3 Determination of cellulose allomorph structure

*X-Ray diffraction technique*

In this study, the distinction of crystalline structure (monoclinic and triclinic) on cellulose used z-Discriminate function developed by Wada *et al.* (2001). The function was built up by separating cellulose $I_\alpha$ and $I_\beta$ by using d-spaces obtained from X-ray analysis (two equatorial d-spacing: 0.59-0.62 ($d_1$) and 0.52-0.55 nm ($d_2$). Value of z> 0 indicated the type of bacteria algae ($I_\alpha$, rich triclinic structure) and z <0 indicated the type of cotton and flax (predominantly $I_\beta$ structure/ monoclinic).

*FT-IR spectroscopy technique*

FT IR (Fourier Transform Infrared Spectrometry analysis) spectra of treated bamboos and controls can be used to strengthen the determination method of cellulose allomorph by direct transmittance using the KBr pellet technique. An amount of 4 mg of bamboo powder was embedded with 200 mg of KBr (potassium bromide) spectroscopy grade in the holder, sealed and then pressed into pellets of 5000 psi. The diameter and thickness of the pellet were approximately 1.3 cm and 0.5 cm. Infrared spectrum patterns were analyzed by using FT IR ABB MB3000. All of the spectras were recorded at a spectral resolution of 16 cm$^{-1}$ and 5 scans were taken per sample with absorption mode in the range of 800-400 cm$^{-1}$. Two absorbance peaks presence i.e 710 and 750-760 cm$^{-1}$, were the characteristics of $I_\beta$ (monoclinic) and $I_\alpha$ (triclinic) allomorphs, respectively (Sassi *et al.* 2000).

### 2.3.4 Crystalite size of cellulose

Determination of cellulose crystallite size used diffraction pattern obtained from 101, 10-1, and 002 lattice planes of bamboo by following the formulation of Ahtee *et al.* (1988).

### 2.3.5 Morphological characteristics (SEM-EDX)

Bamboo morphology prior to and post pretreatment was characterized using a Bruker 133 eV SEM using Carl Zeiss lens. Bamboo samples were installed in the sample (stub) using spunter canter and then scanned at a voltage of 5 kV. EDX was used to determine the percentage of type and element content of the sample. Sample's electron scattering of x-rays were captured by the EDX detector and mapped in the element form based on the received energy difference.

### 2.3.6 Biological degradation pattern by FT IR analysis

Biodegradation pattern of pretreated bamboo was observed by FT IR. Height Peak and area were measured by software HORIZON MB. The relative change in intensity of band as the characteristic of carbohydrates and lignin can be analyzed by the method of Pandeya & Pitman (2003). All spectra were recorded with the spectral resolution of 16 cm$^{-1}$ and 5 scans for recording data. Peak height and area values of lignin associated bands were rationed against carbohydrate reference peaks at 1736, 1378, 1164 and 897 cm$^{-1}$ to provide relative changes in the composition of the structural components relative to each other.

### 2.4 Statistical Analysis

All experiments (chemical composition) were carried out in five and presented in mean and standard deviation. The effects of biological pretreatment on lignin, hemicellulose and cellulose reduction during biological pretreatment were analyzed using Statistical Analysis Software (SAS) program in a 95% confidence level.

## 3. Results and Discussion

### 3.1 The change of chemical content

Fungal pretreatment changed the chemical component composition of betung bamboo (Table 1). This material had high hollocellulose (alpha cellulose and hemicellulose) contents. These fractions are polymers of sugars and thereby a potential source of fermentable sugars to produce ethanol. Teoriticallly, the production potency of sugar yield is 77.28-73.34%, inwhich the conversion factor for reducing sugar is 1.11. Naturally, hollocellulose is resistant to degradation and offer hydrolytic stability, which is mainly due to cross linking lignin via ester and ether linkages (Verma *et al.* 2011). Lignin is the main components in plant cell walls which can limit enzymatic hydrolysis process. Biological pretreatment using white rot fungi can be utilize to facility hydrolytic enzyme to access the ordered crystalline structure of cellulose by splitting the complex bond of cellulose-lignin by extraction or decomposition of the lignin (Zadrazil *et al.*1999).

**Table 1**
Chemical Composition of betung bamboo after pretreatment

| Biological Pretreatment | | Yield (%) | Component (%) | | | | |
|---|---|---|---|---|---|---|---|
| Inoculum loading (%) | Incubation (days) | | Et-ben extractive | alpha cellulose | Lignin | Hemicellulose | Ash |
| 0 | 0 | 100±0.00 | 3.93±1.01 | 44.77±3.39 | 28.01±0.61 | 18.71±3.39 | 3.47±1.61 |
| | 15 | 89.26±7.54 | 3.01±0.35 | 35.26±1.61 | 26.04±0.75 | 20.86±1.27 | 0.77±0.06 |
| 5 | 30 | 93.53±2.90 | 1.44±0.29 | 38.44±1.98 | 20.74±3.17 | 27.49±1.90 | 1.23±0.02 |
| | 45 | 85.29±1.69 | 1.35±0.21 | 33.83±2.01 | 25.18±1.83 | 23.83±1.84 | 0.94±0.07 |
| | 15 | 84.83±2.75 | 2.06±0.64 | 36.75±1.18 | 26.03±0.84 | 19.79±1.71 | 0.67±0.04 |
| 10 | 30 | 93.81±4.40 | 2.08±0.45 | 37.09±0.27 | 24.89±5.01 | 25.66±0.48 | 1.13±0.02 |
| | 45 | 95.69±1.58 | 0.85±0.10 | 38.82±1.99 | 26.53±1.12 | 26.49±0.78 | 1.14±0.04 |

Standard deviations of five replicates

**Table 2**
Component loss of betung bamboo after pretreatment compared to initial mass

| Fungal Pretreatment | | DS | Component loss (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Inoculum loading (%] | Incubation (days) | | Weight | Et-ben extractive | alpha cellulose | Lignin | Hemicellulose | Ash |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 15 | 1.02 | 10.74±7.54 | 23.42±17.28 | 5.51±4.18 | 5.59±2.72 | 32.41±3.94 | 0.92±7.62 |
| | 30 | 8.08 | 6.46±2.90 | 63.40±14.41 | (3.00)±5.29 | 24.27±2.27 | 10.92±6.16 | 13.75±2.36 |
| | 45 | 0.86 | 14.71±1.69 | 65.77±10.56 | 9.35±5.38 | 8.04±1.13 | (27.33)±9.81 | (20.57)±9.28 |
| 10 | 15 | 3.76 | 15.17±2.75 | 47.49±32.04 | 1.51±3.17 | 5.68±3.53 | 35.83±5.54 | 13.52±5.34 |
| | 30 | 16.19 | 6.19±4.39 | 47.16±22.79 | 0.60±0.60 | 9.78±4.22 | 16.83±1.28 | (57.80)±3.02 |
| | 45 | 0.84 | 4.31±1.57 | 78.30±5.06 | (4.05)±5.35 | 3.38±1.48 | (41.62)±4.17 | (46.23)±4.93 |

( ): the value is higher than the untreated samples (the component losses did not happen)
[1]Standard deviations of five replicates; [2]selective value = Lignin loss/Alpha cellulose loss
DS : Delignification Selectivity

Statistical analysis of alpha cellulose indicated that both inoculum loading and interaction between inoculum loading and incubation time gave significant effect to alpha cellulose (p < 0.05 ANOVA). Incubation time and interaction between inoculum loading and incubation time significantly affected the extractives, hemicelluloses and weight loss, while the incubation time was indicated as the only significant factor influencing the lignin.

Fungal pretreatment caused the weight loss of the samples (Table 2) while increasing incubation period tended to decrease the weight loss on 10% inoculum loading. Delignification selectivity is the main parameter to evaluate the delignification effectiveness of fungus treatment. The higher value the more effective process it is and low selectivity value means relatively high cellulose loss during pretreatment. As shown in Table 2, *T. versicolor* pretreatment of both 5 and 10% inoculum loading for 30 days demonstrated better selectivity (8.08 and 16.19) than the other treatments. There was significant increase in the selectivity value with increasing incubation period until 30 days, which indicated the fungus had great selective lignin-degrading ability. The selectivity value of pretreated bamboo decreased after 30 day incubation.This result indicated that *T.versicolor* preferentially degraded lignin only at the early stage of pretreatment and the selectivity turned into non-selective degradation with increasing

pretreatment time. This is in line with the decay of rubber wood by *T.versicolor* and mixed culture of *T.versicolor* and *C. subvermisphora* (Nazarpour *et al.* 2013) and *Pinus radiata* by *Ganoderma austrael* (Ferraz *et al.* 2008). In the lignin attacking activity of fungal enzyme, carbohydrate is also degraded. Among components in bamboo, hemicellulose is the most affected one by fungal pretreatment. As presented in Table 2, the total solid losses were approximately 4.31-15.17%. It might be related to the activity of white rot fungi (WRF) which prefer the degraded-easily accesible-hemicellulose over cellulose to provide nutrient for supporting its growth and methabolism and left cellulose rich (Yu *et al.* 2009). The mycelium grew firstly on the outside of the bamboo and was followed by inside penetration of the strand layer. Partial removal of lignin and hemicellulose destroyed the carbohydrate-lignin complex leading to the disruption of the hydrogen bond between cellulose (Li *et al.* 2010), this activity caused the weight loss after pretreatment.

The main ash components of lignocellulosic materials are calcium, magnesium, potassium and silicon. The minerals are not only incorporated in the cell walls but are also deposited in the lumina of parenchyma cells and libriform fibers (Fengel & Wegener 1989). After fungal pretreatment, there was great decrease in the ash content. Statistical analysis of alpha cellulose loss indicated that only inoculum loading that did not give

significant effect to alpha cellulose loss (p<0.05 ANOVA). Incubation time and interaction between incubation loading and incubation time significantly affected the extractives loss, hemicellulose loss and weight loss while all factors significantly affected lignin loss.

*3.2 Cellulose structure changes of pretreated bamboo*

FTIR spectroscopy was utilized as an analytical tool to qualitatively determine the chemical changes in the surface of pretreated bamboo to complement and understand the XRD investigations.

The infrared spectra in the 800-4000 $cm^{-1}$ region for pretreated bamboo was shown in Fig 1 and 2. The fungus treatment on bamboo only affected the changes in band intensity without changes in functional groups. A strong and broad hydrogen bonded (O-H) stretching absorption was seen at 3420 $cm^{-1}$ (1) and a prominent C-H stretching absorption was seen at around 2920 $cm^{-1}$(2). In the finger print region between 1750 - 800 $cm^{-1}$, the defined peaks were found. The peaks in this region were 1736 $cm^{-1}$ (3) for unconjugated C=O in xylans (hemicellulose), 1635 $cm^{-1}$(4) for absorbed O-H and conjugated C-O as well, both at 1605 $cm^{-1}$(5) and 1512 $cm^{-1}$ (6) for aromatic skeletal in lignin, 1462 $cm^{-1}$ (7) for C-H deformation in lignin and cellulose, 1426 $cm^{-1}$ (8) for C-H$_2$ scissoring motion in cellulose, 1378 $cm^{-1}$ (9) for C-H deformation in cellulose and hemicellulose, 1328 $cm^{-1}$ (10) for C-H vibration in cellulose and C$_1$-O vibration in syringyl derivatives, 1246 $cm^{-1}$ (11) for guaiacyl ring and C-O stretch in lignin and xylan, 1164 $cm^{-1}$ (12) for C-O-C vibration in cellulose and hemicellulose, 1110 $cm^{-1}$ (13) for aromatic skeletal and C-O stretch, 1053 $cm^{-1}$ (14) for C-O stretch in cellulose and hemicellulose, the sharp band at 897 $cm^{-1}$ (15) for C-O-C stretching at the β-glycosidic linkage characteristics in cellulose (Li *et al* 2010) or C-H deformation in cellulose (Pandeya & Pitman 2003), and 833 $cm^{-1}$ (16) for C-H vibration in lignin (Cheng *et al*. 2013).

O-H stretching region at wave number of 3000-3600 $cm^{-1}$ of bamboo spectra was more identical to O-H stretching region from cellulose I than cellulose II.
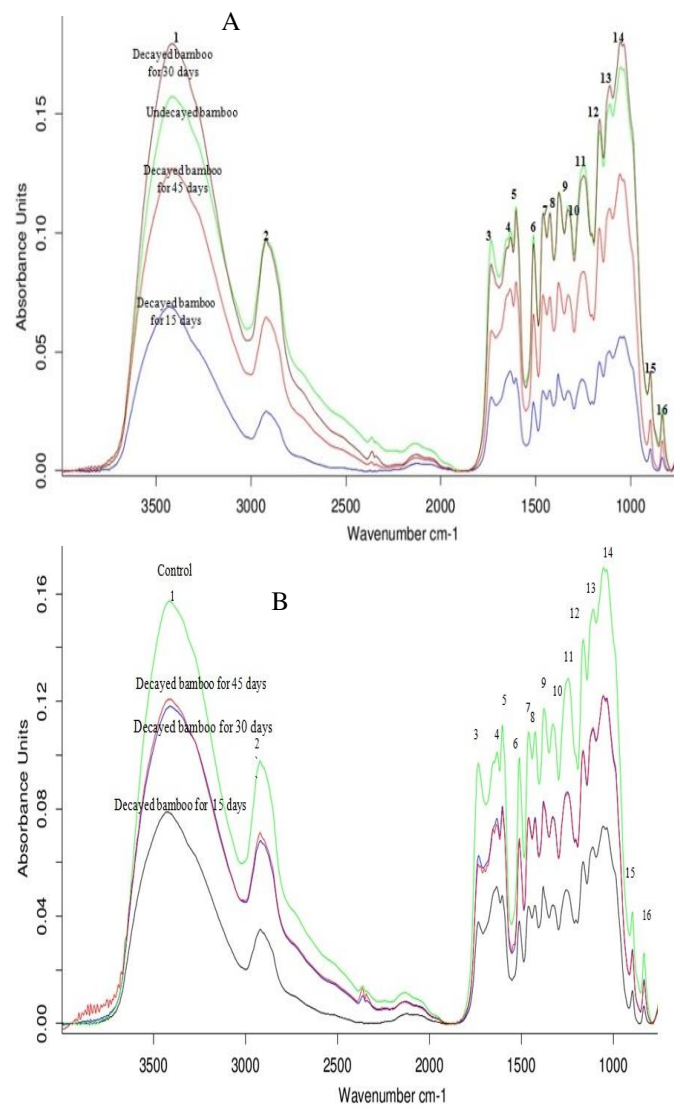


**Fig.1** FTIR spectra of bamboo culms pretreated with *Trametes versicolor* (A) with 5% of inoculum loading and (B) 10% of inoculum loading at different days



**Fig. 2** SEM image of the best biological pretreatment condition

The valance vibration of hydrogen bonding of OH group of cellulose I is the sum of three different hydrogen bonds: intramolecular hydrogen bond of 2-OH···O-6, intramolecular hydrogen bond of 3-OH....O-5 and intramolecular hydrogen bond of 6-OH···O-3 (Oh *et al.* 2005). The absorbency decreased at all times, indicating the decrease of hydrogen bonds. The band at 1635-1640 cm$^{-1}$, which has been attributed to the absorbed water bending vibrations, also decreased after fungal pretreatment. This finding was similar to ionic liquid pretreatment after enzymatic hydrolysis on *Asclepias syriaca* seed floss and poplar seed floss (Spiridon *et al.* 2010). Relative intensities of all bands of decayed bamboo with 10% of inoculum loading were lower than 5% of inoculum loading because of degradation activity of white rot fungi. The IR spectra bands of 1378 cm$^{-1}$, 1735 cm$^{-1}$, 1164 cm$^{-1}$ and 897 cm$^{-1}$ assigned as functional groups of cellulose and hemicellulose showed decrease of intensities compared to control. It indicates that the pretreatment can also lose the hemicellulose and celluloses in this fungal lignin degradation activity. Peak of lignin bands of pretreated samples at 1512 cm$^{-1}$ (aromatic ring stretch) (Pandeya & Pitman 2003) tended to decrease. It is proposed related to lignin release. This fact is in agreement with previous report by Nazarpour *et al.* (2013), indicating the lignin peak intensity decreased significantly compared to carbohydrate and the preferential nature of this fungus. The IR spectra of oil palm empty fruit bunch (OPEFB) pretreated by fungus, *P. florindanus* indicated the attacking linkage of lignin and hemicellulose by this fungus (Faix 1991). This change suggests a split between the benzylic α- and β-carbon atoms by fungal pretreatment (Wistara *et al.* 1999). The decrease in intensity at wave number of 1246 cm$^{-1}$ (guaiacyl of lignin) was greater than at wave number of 1328 cm$^{-1}$ (deformation combination of syringyl and xylan) after fungal treatment of both at 5% and 10% inoculum loading. This suggests that syringyl was more solubilized compared to guaiacyl lignin after fungal treatment. However, the previous research reported by Isroi *et al.* (2012) presented that fungal treatment did not show any absorbance intensity change after fungal treatment by *P. floridanus*s. Lignin and hemicellulose peaks of decayed samples for 15 days were the lowest intensity than the others. This fungus attacked both hemicellulose and lignin simultaneously. This fungus showed more selective activity when samples were exposed to the fungus for 30 and 45 days. The relative amounts of amorphous and crystalline cellulose have earlier been described by Wistara *et al.* (1999) using infrared peak ratios. Lateral order index (LOI), ratio between absorbance at wave number of 1420 and 893 cm$^{-1}$ can also be used to calculate the CI for cellulose I due to the sensivity of the amount of crystalline versus amorphous structure in the cellulose (Oh *et al.* 2005). The LOI showed a linear correlation with the hemicellulose content due to the band at 893 cm$^{-1}$ was assigned to the anomeric carbon group frequency in

cellulose and hemicellulose (Ramos 2003). In this study, the peak pair at 1426 cm$^{-1}$ (crystalline) and 833 cm$^{-1}$(amorphous) was seen for the samples (Fig.1).

Spectra pattern of bamboo decayed at 5% and 10% inoculum loading showed the same trend except for 5% inoculum loading for 30 days. The increase of inoculum loading only affected the decayed samples for 30 days. The decrease of xylan content (1736 cm$^{-1}$) of decayed bamboo for 30 days was less compared to samples exposed for 15 and 45 days (Fig. 1). Intensity band at 1512 cm$^{-1}$ (lignin) of decayed samples for 30 days was appeared the highest. It means that this fungus has preferentially decayed lignin over hemicellulose.

### 3.3 Morphological characteristics of pretreated bamboo

Scanning electron microscopy (SEM) was utilized for visual observation into the morphological structural modification of surface after chosen decayed fungus. Sample which was exposed for 30 days was chosen to observe due to better delignification selectivity than the other treatments. As seen in Fig. 2, *T.versicolor* caused decay on bamboo cell wall compared to undecayed sample (control). The original sample showed compact rigid particle structure while the pretreated samples had more fragile and distorted bundles. Disruption of cell wall matrix including connection between carbohydrates and lignin as well as depolymerizing and solubilising hemicellulose polymers were pretreatment effect (Ramos 2003). Lignin degradation caused by fungal pretreatment can increase pore sizes in the substrate and provide a more accessible surface area to cellulase. Photomicrographs of oil palm empty fruit bunch (OPEFB) showed that some of silica bodies was removed from the strand surface and left empty holes at the bottom of silica-bodies creatures. The surface of fungal pretreated OPEFB are rugged and broken faced, partially followed by the mycelium growth on the outside and penetrate inside the strand (Isroi *et al.* 2010). Taniguchi *et al.* (2010) reported that the partial network collapse of the rice straw fibers happened with a simultaneous increase in surface area on the biological pretreatment using *Pleurotus ostreatus*. The cellulose digestibility can be potentially enhanced by preferential degradation of lignin (Nazarphour *et al.* 2013). The fibers were partially separated in biological pretreatment, presumably due to cleavage of some ether bonds in lignin and lignin-carbohydrate linkages. There is no difference in decayed rate between 5 and 10% of inoculum loading for 30 days. Closer observation in ultra structure level is required to study degradation changes in cell walls due to lignin degradation activity of the fungus. The degradation of cell wall had possibly happened and the mechanism of this process was stated by Akhar *et al.* (1998). The fungi might colonize in all planes; the decay captured the nutrient and then colonizes it to distribute the fungal mycelium.
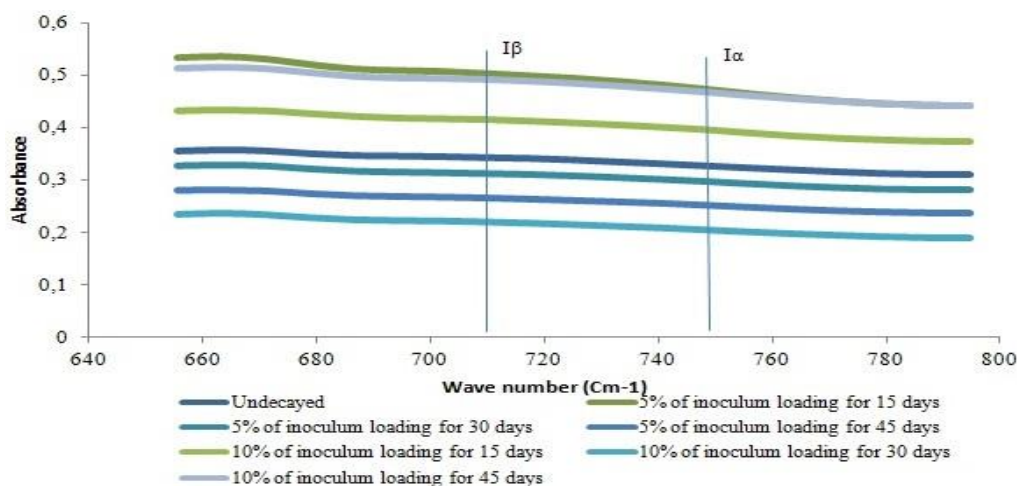
**Fig. 3** FTIR derivative spectra at wave number of 750-760 cm-1 ($CH_2$ vibration in cellulose $I_\alpha$) and 710 cm$^{-1}$($CH_2$ vibration in cellulose $I_\beta$)

**Table 3**
Element weight percentage of pretreated bamboo

| Element | Undecayed bamboo wt.% | 5% of inoculum loading wt.% | 10% of inoculum loading wt.% |
|---|---|---|---|
| Carbon (C) | 44.65±13.7 | 42.30±13.0 | 42.30±13.0 |
| Oxygen (O) | 44.71±13.8 | 55.72±17.2 | 55.72±17.2 |
| Fluorine (F) | 9.05±3.2 | | |
| Magnesium (Mg) | 0.84±0.1 | 1.11±0.1 | 1.11±0.1 |
| Aluminium (Al) | 1.11±0.1 | 0.87±0.1 | 0.87±0.1 |
| Silicon (Si) | 0.87±0.1 | | |
| Total | 100 | 100 | 100 |

**Table 4**
Crystalline allomorph of pretreated bamboo

| Inoculum loading (%) | Incubation time (days) | Crystallite allomorph | | | Crystal allomorph |
|---|---|---|---|---|---|
| | | d (101) nm | d (10-1) nm | z | |
| 0 | 45 | 0.566041 | 0.534862 | -73.13 | $I_\beta$ |
| 5 | 15 | 0.588426 | 0.522358 | -23.96 | $I_\beta$ |
| | 30 | 0.615201 | 0.521747 | 21.92 | $I_\alpha$ |
| | 45 | 0.615201 | 0.546641 | -0.53 | $I_\beta$ |
| 10 | 15 | 0,588426 | 0,566041 | -63.36 | $I_\beta$ |
| | 30 | 0,616903 | 0,520533 | 25.89 | $I_\alpha$ |
| | 45 | 0,602733 | 0,535504 | -11.59 | $I_\beta$ |

Element percentage presence and their types are recapitulated in Table 4 based on EDX (Energy Dispersive X-ray Spectroscopy) analysis. Fluorine and silicon were not found after samples treated by this fungus, meanwhile the other elements still existed with different level of elements. Carbon and oxygen dominated over the other elements. It can be understood due to bamboos were included as lignocellulosic materials comprised of carbon, hydrogen and oxygen which form major structural components such as carbohydrate and lignin polymer. Inorganic constituents were contained in the ash, the residue after burning the organic matter, such as magnesium and aluminum which were still founded in decayed samples in small quantities while fluorine and silicon lost from samples. This fungus treatment caused carbon reduction that might be used for supporting its growth.

Crystalline allomorph of pretreated bamboo was represented in Table 2 and Fig. 3 studied by two methods i.e. XRD and FTIR analysis. It is well known that the native cellulose consists of two distinct crystalline modifications i.e. $I_\alpha$ (one-chain triclinic) and $I_\beta$ (two-chain monoclinic cells), whose fractions vary depending on the origin of cellulose sample (Sugiyama *et al*. 1991). Z-discriminant < 0 indicated the $I_\beta$ type allomorph, and Z > 0 was characterized as $I_\alpha$ type. monoclinic ($I_\beta$) cellulose is thermodynamically stable compared to triclinic ($I_\alpha$), due to it is not only dense, but also invariably tends to be final product in heat annealing of all celluloses (Sassi *et al*. 2000). $I_\beta$ structure of cellulose transformed to triclinic structure on the fungus treatment for 30 days. Z-discriminant determination was performed based on 101 and 10-1 lattice planes of bamboo samples. Triclinic structure was expected to give a better effect on hydrolysis performance due to the $I_\alpha$ phase was more degradable than $I_\beta$ (Wada & Okano 2001). Cellulose $I_\alpha$ is meta-stable and more reactive than $I_\beta$ (O'Sullivan 1997). In addition, cellulase of *Tricoderma viride* preferentially degraded the $I_\alpha$ form compared to $I_\beta$ form and residual micro crystals became $I_\beta$-rich along with time (Hayashi *et al*.1998a, 1998b). $I_\alpha$ form reactivity to acetylation was higher than that of $I_\beta$ phase

(Sassi *et al*. 2000) and this phase is more abundant at the surface of microcrystals (Hayashi *et al*. 1998a,b).

The peaks at wave numbers of around 750-760 cm$^{-1}$ and 710 cm$^{-1}$ were assigned to rocking vibration CH$_2$ in cellulose I$_\alpha$ and cellulose I$_\beta$, respectively. As can be seen in the Fig.3, there was no peak in wave number of 750-760 cm$^{-1}$ and 710 cm$^{-1}$. Crystalline allomorph changes was only seen based on XRD analysis, but not in FTIR analysis.

### 3.4 Biodegradation pattern

Biodegradation patterns of bamboo during biological pretreatment with *T.versicolor* were evaluated by FTIR analysis. To fully understand the biodegradation patterns of pretreated bamboo, a detailed FTIR spectroscopic analysis based on Pandeya's analysis method was performed (Pandeya & Pitman 2003). Table 5 showed relative changes in the intensities of lignin peaks at 1512 cm$^{-1}$ against carbohydrate peaks at 1736, 1373, 1165, 897 cm$^{-1}$ which were calculated by peak heights and areas. These four carbohydrate peaks assigned, respectively, the unconjugated derived from aromatic framework (aromatic skeletal) in lignin (Fig.4).

Four peaks of carbohydrates for the unconjugated bonds are C=O in xylan, C-H deformation in cellulose and hemicellulose, C-O-C vibration in cellulose and hemicellulose and C-H deformation or C-O-C stretching at the β-glycosidic linkage characteristics in cellulose (Pandeya & Pitman 2003). Generaly, there is an increase in lignin/carbohydrate ratio of bamboo pretreated by *T. versicolor* with increasing incubation time, which indicated the fungus had unselective lignin-degrading ability. The previous study by Zhang *et al*. (2007) in bamboo culm and Ferraz *et al*. (2000) in *Pinus radiata* reported that *T. versicolor* G20 and *Gonoderma australe* preferentially degrade lignin only at the early stage pretreatment and the selectivity turned into non selective degradation at prolonged incubation time. However, this ratio tended to decrease significantly with increasing pretreatment time on the bamboo culm pretreated by *E. taxodii* 2538, which indicated its great selective lignin-degrading ability. In this study, only ratio

of I$_{1512}$/I$_{897}$ of 5% inoculum loading which indicated this trend while the other ratios showed inverse trend. The increase of the other ratios indicated that hemicellulose and cellulose degradation levels were higher.

### 3.5 Cellulose crystallinity index

Cellulose crystallinity index and lateral order index (LOI) of pretreated bamboo was tabulated in Table 6. Naturally, cellulose chains contain both crystalline (ordered) and amorphous (less ordered) regions. Crystallinity structure of cellulose can inhibit the sugar release in lignocellulosic conversion. Crystallinity index (CI), describing the relative amount of crystalline material in cellulose, is one of the most important parameters, which can be measured by XRD, solid-state $^{13}$C NMR, infrared (IR) spectroscopy and raman spectroscopy. The minimum intensity of XRD diffraction of the (101) lattice peak at 2θ equal to 18$^o$ represents the amorphous cellulose regions and the maximum intensity of the (002) lattice peak at 2θ equal to 22$^0$ represents crystalline cellulose regions. The index rises along with increasing ratio of crystalline to amorphous parts. Pretreatment is also able to change the crystallinity index (Chang & Holtzapple 2000). This index of pretreated bamboo was higher than that of control. The index increasing can be caused by cleavage of amorphous fractions of cellulose such as lignin and hemicellulose occuring during pretreatment compared to that of crystalline fraction. The high crystallinity of pretreated sample indicates that the fungus was able to remove lignin and hemicellulose components of bamboo compared to degrading cellulose. This finding was supported by previous research (Evans *et al*.1995, Nazarphour *et al*. 2013, Highley *et al*. 1989, Kleman-Leyer *et al*.1992). After 30 days, crystallinity index bagan to decrease at 5% of inoculum loading, possibly due to continuing fungal attack on the crystalline cellulose only after degradation of the more readily available amorphous nutrient sources. In the 10% of inoculum loading, the crystallinity index increased after 30 day incubation. However, this pretreated sample index remained above the level of the control.
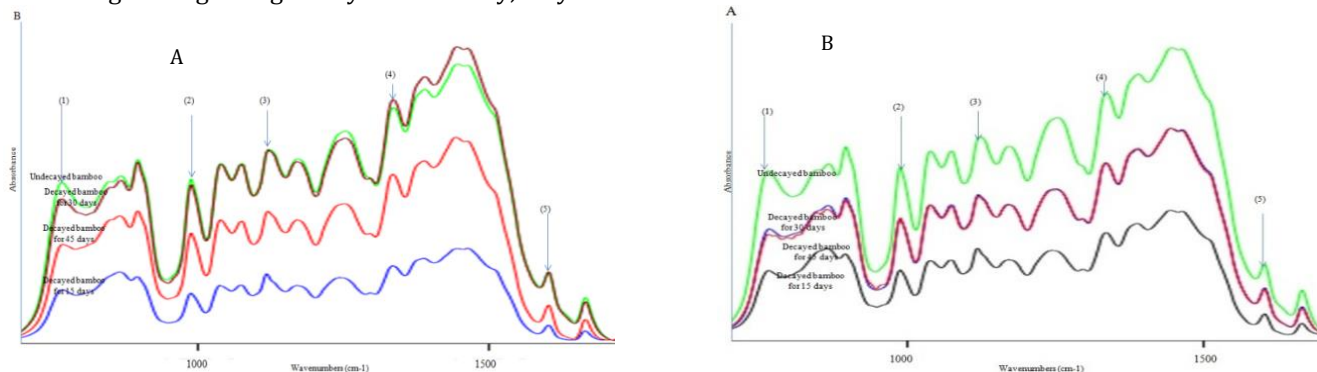


**Fig. 4** FTIR spectra of bamboo culms pretreated with *Trametes versicolor* (A) 5% of inoculum loading and 10% of inoculum loading (B) for different days. (1) 1736 cm$^{-1}$, (2) 1512 cm$^{-1}$, (3) 1373 cm$^{-1}$, (4) 1165 cm$^{-1}$, (5) 897 cm$^{-1}$.

The digestibility of OPEFB after pretreatment has an inverse correlation with LOI value. LOI value of pretreated samples is lower than untreated ones except on 10% of inoculum loading for 30 days (Table 6). It indicated that fungal pretreatment can improve cellulose surface assesibility and theoretically support more efficient cellulose hydrolysis on the treated cellulose materials. The fungal preferably degraded the easily accesible hemicellulose over cellulose to provide nutrient for supporting its growth and metabolism and left cellulose rich (Yu *et al.* 2009). The transformation of crystalline structure of cellulose into amorphous form increase the LOI value. The ionic liquid pretreatment on cellulose caused this conversion (Li *et al.* 2010). Another infrared ratio was calculated at $A_{3308}/A_{1330}$, which known as hydrogen-bond intensity (HBI) (Oh *et al.* 2005, Siroky *et al.* 2010).

Crystallite size of cellulose in bamboo varied at lattice planes (101), (10-1) and (002) (Fig.5) in which lattice plane in (002) was identified as crystalline (cellulose) region, while (001) and (10-1) were amorphous (lignin and hemicellulose) region. The highest crystallite size of cellulose in untreated bamboo at 002 lattice plane was determined at 5.59 nm (Table 7). This value was higher than those of hemp bast fiber, wheat straw and cotton linter reported in previous studies (Gumuskaya & Usta 2002, Gumuskaya *et al.* 2003). There was no significant changes in crystallite size at 002 lattice plane of pretreated bamboo except on samples pretreated with 5% inoculum loading for 45 days. Crystallite size at 040 lattice plane showed crystalline area length. The treatment could reduce the crystalline length of samples except on samples pretreated with 5% of inoculum loading for 45 days.

## 4. Conclusions

We investigated how the biological pretreatment with *T.versicolor* affected the betung bamboo characteristics. Incubation period for 30 days showed the greatest delignification selectivity. Syringyl of lignin was more affected than guiacyl moties and the hydrogen bond also changed after pretreatment indicated by decreasing of band intensity. The treatment increased the crystallinity index with the amorphous fraction cleavage during fungus incubation. The pretreated samples had more fragile and distorted bundles confirmed by SEM. In addition, the parts of element samples (fluorine and silicon) were lost after pretreatment. Crystalline allomorph showed that $I_\beta$ (monoclinic) structure of cellulose has transformed into $I\alpha$ (triclinic) structure on the fungus pretreatment for 30 days.

**Table 5**

Ratio of intensity of lignin-associated band with carbohydrate bands for pretreated bamboo

| Inoculum loading (%) | Incubation (days) | Relative intensities[a] of aromatic skeletal vibration ($I_{1512}$) againts typical bands for carbohydrates | | | |
|---|---|---|---|---|---|
| | | $I_{1512}/I_{1736}$ | $I_{1512}/I_{1373}$ | $I_{1512}/I_{1165}$ | $I_{1512}/I_{897}$ |
| 0 | 0 | 1.02 (0.91) | 1.05(1.15) | 1.02(1.2) | 1.33(1.25) |
| 5 | 15 | 1.14(1.02) | 0.89(0.98) | 0.80(0.94) | 8.00(1.25) |
| | 30 | 1.25(1.03) | 1.00(1.05) | 0.83(1.00) | 5.00(1.29) |
| | 45 | 1.42(1.06) | 1.11(1.00) | 1.00(1.03) | 1.43(1.24) |
| 10 | 15 | 1.04(1.08) | 1.02(1.08) | 0.98(0.96) | 1.28(1.3) |
| | 30 | 1.67(1.08) | 1.67(1.00) | 0.83(0.96) | 5(1.29) |
| | 45 | 0.97(0.26) | 0.78(1.00) | 0.70(0.97) | 7(1.25) |

Relative intensities were calculated using peak heights (out the parentheses) and areas (in the parentheses)

**Table 6**

Lateral Order Index and degree of cellulose crystallinity of pretreated bamboo

| Inoculum loading (%) | Incubation (days) | Degree of crystallinity (Xc) | | | Lateral Order Index(LOI) | | |
|---|---|---|---|---|---|---|---|
| | | Crystalline (Fc) | Amorphous (Fa) | Xc | $A_{1427}$ (Crystalline) | $A_{897}$ (Amorphous) | LOI |
| 0 | 0 | 0.66 | 1.51 | 30.43 | 0.42 | 0.33 | 1.27 |
| 5 | 15 | 0.76 | 1.44 | 34.58 | 0.60 | 0.48 | 1.25 |
| | 30 | 0.98 | 1.58 | 38.39 | 0.38 | 0.31 | 1.23 |
| | 45 | 0.47 | 1.00 | 32.00 | 0.31 | 0.25 | 1.24 |
| 10 | 15 | 0.99 | 1.72 | 36.62 | 0.50 | 0.40 | 1.25 |
| | 30 | 0.69 | 1.57 | 30.83 | 0.27 | 0.21 | 1.29 |
| | 45 | 0.93 | 1.55 | 37.48 | 0.59 | 0.48 | 1.23 |

**Table 7**

Crstallite size of pretreated bamboo

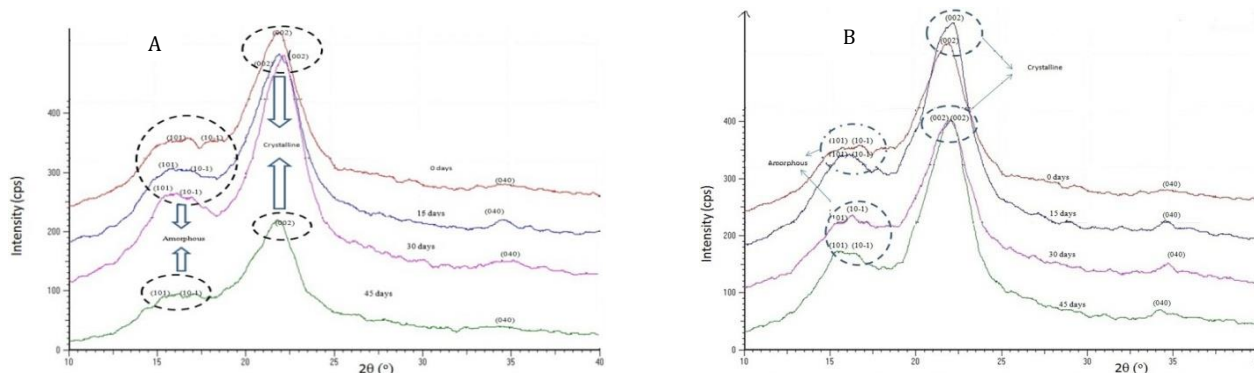| Inoculum loading (%) | Incubation (days) | Crystallite size | | | |
|---|---|---|---|---|---|
| | | D(101) nm | D (10-1) nm | D (002) nm | D(040) nm |
| | 0 | 5.46 | 8.71 | 5.59 | 129.39 |
| 5 | 15 | 8.04 | - | 5.47 | 17.45 |
| | 30 | 17.79 | - | 5.57 | 64.70 |
| | 45 | - | 23.40 | 7.40 | 258.83 |
| 10 | 15 | 7.96 | 4.30 | 5.64 | 25.88 |
| | 30 | 13.84 | - | 5.61 | 25.46 |
| | 45 | 10.68 | - | 5.47 | 19.39 |

**Fig. 5** XRD spectra of bamboo pretreated with *Trametes versicolor* (10% of inoculum loading (A) 5% of inoculum loading for different days (B) for different days

## Acknowledgements

## References

Ahtee, M., Hattula, T., Mangs, J., & Paakkari, T. (1988) An X-ray Diffraction Method for Determination of Crystallinity of Wood Pulp. *Paperi Ja Puu*, 8, 475-480.

Akhtar, M.,Scott, G.M., Swaney, R.E., & Kirk, T.K. (1998) *Enzyme Applications in Fiber Processing*, American Chemichal Society,Washington,DC.

Bohn, A., Fink, HP., Ganster, J., and Pinnow, M. (2000) X-Ray Texture Investigations of Bacterial Cellulose. *Macromolecular Chemistry and Physic,* 201, 1913–1921.

Chang VS.,& Holtzapple, M.T. (2000) Fundamental Factors Affecting Bio-mass Enzymatic Reactivity. *Appl Biochem Biotechnol*, 84, 5-37.

Cara,C., Ruiz, E., Oliva, J.M., Saez, F., Castro, E. (2008) Conversion of Olive Tree Biomass into Fermentable Sugars by Dilute Acid Pretreatment and Enzymatic Saccarification. *Bioresource Technology,* 99, 1869-1876

Cheng,D., Jiang, S.,& Zhang, Q. (2013) Effect of Hydrothermal Treatment with Different Aqueous Solutions on the Mold Resistance of Moso Bamboo with Chemical and FTIR Analysis *BioResources* 8(1):371-382.

Dransfield,S., & Widjaja, E.A. (1995) Plant Reseources of South-East Asia No.7.Bamboos, Leiden:Backhuys Publishers, pp.189

Eriksson, K.E., Blanchette, R.A., Ander, P. (1990) *Microbial and Enzymatic Degradation of Wood and Wood Components*, 1st edition,Springer Verlag, New York.

Evans, R., Newman, R., & Roick, U. (1995) Changes in Cellulose Crystallinity during Kraft Pulping. Comparison of Infrared, X-ray Diffraction and Solid State NMR Results*, Holzforschung*,49, 498–504.

Ferraz, A., Parra, C., Freer, J., Baeza, J., & Rodrı´guez, J. (2000) Characterization of White Zones Produced on pinus Radiata wood Chips by Ganoderma Australe and *Ceriporiopsis Subvermispora*,World Journal of Microbiology and Biotechnology,16, 641–645.

Ferraz, A., Guerra,A., Mendoca, A.R., Masarin, F., Vicentim, M.P., Aguiar A., & Pavan, P.C. (2008) Technological Advances and Mechanistic Basis for Fungal Biopulping, *Enzyme and Microbial Technology,* 43, 178-185

Fatriasari, W., & Hermiati, E. (2008) Analisis Morfologi Serat dan Sifat Fisis-Kimia pada Enam Jenis Bambu sebagai Bahan Baku Pulp dan Kertas. *Jurnal Ilmu dan Teknologi Hasil Hutan*,1(2), 67-72.

Fatriasari, W., Ermawar, R.A., Falah, F, Yanto, D.H.Y., Adi, D.T.N., Anita, S.H., & Hermiati, E. (2011) Kraft and Soda Pulping of White rot

Pretreated Betung Bamboo, *Jurnal Ilmu dan Teknologi Kayu Tropis,* 9(1), 42-55.

Falah, F., W. Fatriasari, R.A. Ermawar, D.T.A.N., & Hermiati, E. (2011) Effect of Corn Steep Liquor on Bamboo Biochemical Pulping Using *Phanerochaete chrysosporium*, *Jurnal Ilmu dan Teknologi Kayu Tropis* 9(2): 111-125.

Focher, B., Pala, M.T., Canetti, M., Torri, G., Cosentino, C.,&Gastaldi, G. (2001) Structural Differences Between Non-Wood Plant Cellulose: Evidence from Solid State NMR, Vibrational Spectroscopy and X-Ray Diffractometry. *Ind Crops Products*,13, 193-2008.

Fengel, D., & Wegener, G. (1989) *Wood. Chemistry, ultrastructure, reactions*, Walter de Gruyter,Berlin & New York.

Faix, O. (1991) Classification of Lignins from Different Botanical Origins by FT-IR Spectroscopy, *Holzforschung*,45, 21–27.

Guillén, F., Muñoz, C., Gomez-Toribio, V., Martínez, AT., and Martínez, M.J. (2000) Oxygen Activation during Oxidation of Methoxyhydroquinones by Laccase from *Pleurotus Eryngii, Appl. Environ. Microbiol.*,66, 170–175.

Gelbe, M., & Zacchi, G. (2007) Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production. *Adv.Biochem.Eng./Biotechnol*,108, 41-65.

Giles, R.L., Galloway, E.R., Elliott, G.D., & Parrow, M.W. (2011) Oxygen Activation during Oxidation of Methoxyhydroquinones by Laccase from *Pleurotus Eryngii. Bioresource Technology* 102, 8011-8016.

Gumuskaya,E., & Usta, M. (2002) Crystalline Structure Properties of Bleached and Unbleached Wheat Straw (*Triticum aestivum* L.) Soda-Oxygen Pulp.*Turk J.Agric For*, 26, 247-252

Gumuskaya, E., Usta, M., & Kirci, H. (2003) The Effects of Various Pulping Conditions on Crystalline Structure of Cellulose in Cotton Linters. *Polymer Degradation and Stability,* 81, 559-564

Messner, K., & Srebotnik, E. (1994) Biopulping: An Overview of Developments in An Environmentally Safe Paper Making Technology.*FEMS Microbiology Reviews,* 13,351-364.

Gumuskaya E, & Usta, M. (2006) Dependence of Chemical and Crystalline Structure of Alkali Sulfite Pulp on Cooking Temperature and Time. *Carbohydrate Polymers*, 65, 461–468.

Gratani, L., Crescente, M.F.,Fabrini, L.G., and Digiulio, E. (2008) Growth Pattern and Photosynthetic Activity of Different Bamboo Species Growing in the Botanical Garden of Rome. *Flora*, 20, 77-84

Hakala T.K., Lundell, T., Galki,S., Maijala, P., Kalkkinen, N., &Hatakka, A. (2005) Manganese Peroxidases, Laccases and Oxalic Acid from the Selective White-Rot Fungus *Physisporinus Rivulosus* Grown On Spruce Wood Chips. *Enzyme and Microbial Technolog,* 36**,** 461-468.

Hayashi,N., Ishihara,M., Sugiyama,J., & Okano,T. (1998a) The Enzymatic Susceptibility of Cellulose Microfibrils of the Algal-Bacterial Type and Cotton-Ramie Type, *Carbohydrate Research*,305, 109-116.

Hayashi,N., Ishihara,M., Sugiyama,J., & Okano,T. (1998b) Selective Degradation of Cellulose Ia Component in *Cladophora* Cellulose with *Trichoderma viride* Cellulose, *Carbohydrate Research,*05:\, 261-269.

Highley,T., Kirk,T.,Ibach, R. (1989) Properties of Cellulose Degraded by the Brown-Rot Fungus. International Research Group on Wood Preservation,Stockholm, Sweden.

Kleman-Leyer, K., Agosin, E., Conner, A.H., & Kirk,T.K (1992) Changes in Molecular Size Distribution of Cellulose during Attack by White Rot and Brown Rot Fungi. *Appl.Environ.Microb.*58:1266–1270.

Isroi, I., Millati, M.M., Syamsiah, R., Cahyanto, M.N.,Niklasson, C., & Taherzadeh, M.J (2012) Structural Changes of Oil Palm Empty Fruit Bunch (OPEFB) after Fungal and Phosphoric Acid Pretreatment, *Molecules,* 17, 14995-15012.

Kant, P. (2010) Should Bamboos and Palms be Included in CDM Forestry Projects?, IGREC Working paper,No.IGREC-07:2010, Institute of Green Economy, New Delhi.

Kirk, T.K., & Chang, H-M. (1981) Potential Application of Bio-lignolytic Systems. *Enzyme and Microbial Technology* 3, 189-196

Li,X., Ximenes, E., Kim,Y., Slinger, M., Meilan,R., Ladisc, M.& Chapple,C. (2010) Lignin Monomer Composition Affects *Arabidopsis* Cell-Wall Degradability after Liquid Hot Water Pretreatment *Biotechnology for Biofuels* **3**:27.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., & Nilvebrant, N. (1999) The Generation of Fermentation Inhibitors during Dilute Acid Hydrolysis of Softwood. *Enzyme and Microbial Technology*, 24, 151–159.

Lobovikov, M., Paudel, S., Piazza, M., Ren, H., & Wu, J. (2007) World Bamboo Resources. A Thematic Study Prepared in the Framework of the Global Forest Resources Assessment 2005.Non Wood Forest Products 18, Food and Agricultural Organization of the United Nations, Rome.pp. 73

Mokushitsu Kagaku Jiken Manual (2000) Japan Wood Research Society Publisher.

Mosier, N., Wyman, C., Dale, B., Elander, R., Holtzapple, Y.Y.L.M., & Ladisch, M. (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technol*, 96, 673-686.

Nasendi, B.D., (1995) Growth Pattern and Photosynthetic Activity of Different Bamboo Species Growing in The Botanical Garden of Rome. A paper prepared for IV-International Bamboo Congress/Research Workshop, 19-22 June 1995 in Ubud, Bali, Indonesia, Forest Products and Forestry Socio-Economic Research and Development Center, Bogor

Nazarpour,F., Abdullah, D.K., Abdullah, N., & Zamiri, R. (2013) Evaluation of Biological Pretreatment of Rubberwood with White Rot Fungi for Enzymatic Hydrolysis, *Materials* 6, 2059-2073

Oh, S.Y., Yoo, D.I., Shin, Y., & Seo, G. (2005) FTIR Anaysis of Cellulose Treated with Sodium Hydroxide and Carbon Dioxide *Carbohydrate Research* 340(3), 417-428.

O'Sullivan (1997) A cellulose structure slowly unrevels, *Cellulose* 4, 173-207

Pandeya, K.K. and Pitman, A.J. (2003). FT IR Studies of the Changes in Wood Chemistry Flowing Decay by Brown-Rot and White-Rot Fungi. *International Biodeterioration & Biodegradation,* 52, 151-160.

Ramos, L.P. (2003) The Chemistry Involved in The Steam Treatment of Lignocellulosic Materials *Quim Nova*, 26, 863-871.

Sassi, J-F., Tekely, P., & Chanzy, H. (2000) Relative Susceptibility of the *Iα* and *Iβ* Phases of Cellulose Towards Acetylation *Cellulose*,7, 119-132.

Spiridon,I., Teaca, C-A., & Bodirlau, R. (2010) Structural Changes Evidenced by FTIR Spectroscopy in Cellulosic Materials after Pre-Treatment with Ionic Liquid and Enzymatic Hydrolysis *BioResources* 6(1), 400-413.

Sun Y., & Cheng, J. (2002) Hydrolysis of Lignocellulosic Materials for Ethanol Production: A Review, *Bioresource Technology***,** 83**,** 1-11.

Scurlock, J.M.O., Dayton, D.C., and Hames, B. (2000) Bamboo: an Overlooked Biomass Resource?.*Biomass and Bioenergy*, 19, 229–244.

Sathitsuksanoh, N., Zhu, Z., Templeton, N., Rollin, J., Harvey, S., & Zhang, Y.H.P. (2009) Saccarification of a Potential Bioenergy Crop, *Phragmites australis* (common reed), by Lignocellulose Fractionation Followed by Enzymatic Hydrolysis at Decreased Cellulose Loadings. *Ind.Chem.Res.,* 48, 6441-6447.

Sugiyama,J., Persson,J., & Chanzy, H. (1991) Combined Infrared and Electron Diffraction Study of the Polymorphism of Native Celluloses, *Macromolecules*,24, 2461-2466.

Siroky, J., Blackburn,R.S., Bechtold,T., Taylor,J., & White,P. (2010) Attenuated Total Reflectance Fourier-Transform Infrared Spectroscopy Analysis of Crystallinity Changes in Lyocell Following Continuous Treatment with Sodium Hydroxide *Cellulose,*17, 103-115.

Tanaka T., Fujita, M., Takeuchi, A., Suzuki,Y., Uesugi, K., Ito, K., Fujisawa, T., Doi, Y., & Iwata, T. (2006) Formation of Highly Ordered Structure in Poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3-hydroxyvalerate] High-Strength Fibers. *Macromolecules,* 39, 2940–2946.

Taniguchi, M., Takahashi, D., Watanabe, D., Sakai, K., Hoshino,K., Kouya,T., & Tanaka, T. (2010) Effect of Steam Explosion Pretreatment on Treatment with *Pleurotus Ostreatus* for the Enzymatic Hydrolysis of Rice Straw, *J. Biosci. Bioeng.*,110, 449–452.

Verma, A., Kumar, S., Jain, P.K. (2011) Key Pretreatment Technologies on Cellulosic Ethanol Production. *Journal of Scientific Research*, 55, 57-63.

Wistara, N., Zhang, X. J., & Young, R.A. (1999) Properties and treatments of pulps from recycled paper. Part II. Surface Properties and Crystallinity of Fibers and Fines *Cellulose*,6(4), 325-348.

Widjaja, E.A., (2011) The Utilization of Bamboo: at Present and for The Future. Proceedings of International Seminar Strategies and Chalanges on Bamboo and Potential Non Timber Forest Products (NTFPs) Management and Utilization, Puslitbang Hasil Hutan, Bogor-Indonesia

Wada, M., Okano,T., & Sugiyama, J. (2001) Allomorphs of Native Crystalline Cellulose I Evaluated by Two Equatorial *d*-Spacings *J.Wood Science*,47, 124-128.

Wyman, C.E. (2007) What Is (and Is Not) Vital to Advancing Cellulosic Ethanol. *Trends in Biotechnology* 25(4), 153-157.

Wan,C., & Li,Y. (2010) Microbial Pretreatment of Corn Stover with *Ceriporiopsis subvermisphora* for Enzymatic Hydrolysis and Ethanol Productiaon. *Bioresource Technology* 101, 6398-6403.

Wada, M., & Okano,T. (2001) Localization of Iα and Iβ Phases in Algal Cellulose Revealed by Acid Treatments, *Cellulose* 8, 183-188.

Yu H, Guo G, Zhang, X., Yan K, & Xu C. (2009) The Effect of Biological Pretreatment with the Selective White-Rot Fungus *Echinodontium Taxodii* on Enzymatic Hydrolysis of Softwoods and Hardwoods, *Bioresource Technol* 100, 5170-5175

Zhang,Y.-H.P. (2008) Reviving the Carbohydrate Economy Via Multi-Product Biorefineries, *J.Ind.Mucrobiol.Biotechnol.*,5, 367-375

Zhao, H.K., Kwak, J.H., Wang, Y., Franz, J.A., White, J.M., & Holladay, J.E. (2006) Effects of Crystallinity on Dilute Acid Hydrolysis of Cellulose By Cellulose Ball-Milling Study. *Energy & Fuels,* 20(2), 807-811.

Zhang, X., Xu, C., & Wang, H. (2007) Pretreatment of Bamboo Residues with *Coriolus versicolor* for Enzymatic Hydrolysis *Journal of Bioscience and Bioengineering,* 104 (2), 149-151.

Zadrazil, F., Permana, I.G., & Carsten in der Wiesche (1999) Is the Conversion of Lignocellulosics into Feed with White Rot Fungi Realizable? Practical Problems of Scale Up and Technology Transfer. Mushroom Sci 15, Session: Sustainable Technology Development in Animal. Agriculture Institut für Pflanzenernährung und Bodenkunde, Bundesforschungsanstalt für Landwirtschaft,Bundesallee 50, 38116 Braunschweig, Germany, pp. 919-928.