FIBER QUALITIES OF PRETREATED *BETUNG* BAMBOO (*Dendrocalamusasper*) BY MIXED CULTURE OF WHITE-ROT FUNGI WITH RESPECT TO ITS USE FOR PULP/PAPER

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ABSTRACT

Previous research on anatomical structures of pretreated large (*betung*) bamboo (*Dendrocalamusasper*) using single culture of white-rot fungi has been investigated, which revealed that the pretreatment caused the decrease in the Runkel ratioas well as the coefficient rigidity and the increase in the flexibility ratio of their corresponding bamboo fibers. However, there is no study reported on the anatomical structure changes of them caused by pretreatment using mixed culture of white-rot fungi. This paper reports the results of the research on paper/pulp quality after different treatments. Pretreatment that used *Trametes versicolor* fungi and lasted for 45 days inflicted intensive fiber damages compared with those of untreated bamboo (control). Fresh and barkless large (*betung*) bamboo chips of 2 year's old, and 1.6 cm in length, were inoculated by 10% of mixed culture of white-rot fungi inoculums stock for 30 and 45 days in room temperature. There were four treatment groups of mixed culture, i.e *T. versi color and P. ostreatus (TVPO); P. ostreatus* and *P. chrysosporium (POPC); P. chrysosporium and T.versi color (PCTV); and P.chrysosporium, T.versicolorand P.ostreatus (TVPCPO).* After the inoculation period, the chips were macerated into separate fibers using Scultze method to analyze the fiber dimension and its derived values. The fibers were then observed regarding their macro and microscopic structures by optical microscope. Mixed culture pretreatment of white-rot fungi accelerated improvement of fiber morphology and fiber derived value characteristics, except for Muhlsteph ratio. The fiber derived values oftreated bamboo tended to improve compared to those of untreated bamboo, there by requiring milder pulping conditions. Accordingly, the treated bamboo would indicatively produce a good quality pulp (grade I) based on FAO and LPHH (Forest Product Research Report) requirements. Co-culture treatment using *P. chrysosporium* and *P. ostreatus* for 45 days produced the best fiber dimension and its derived value properties. The fungi hypae colonized on the surface area of bamboo followed by mycelium penetration into substrate (bamboo-inner structure). The partial degradation caused by delignification indicatively attributed to the fungi activity was shown in the macroscopic images.

Keywords: *Betung* bamboo, mixed culture, white rot fungi, fiber dimension,fiber-derived value

I. INTRODUCTION

Bamboos typify as plants belonging to graminae, which afford very efficient photosynthesis ability as shown by the high production of lingo-cellulosic biomass (20-40 tons/ha/year), and this production is about 7-30% higher than that of woody plants (Kant, 2010). These lignocellulosic materials as commonly produced by spermatophytaor (seed-bearing) plants (including woody softwood, woody hardwood, and bamboo monocot) are widely used for the production of pulp and paper, fiberboard, textile, food, methane, lactic acid, construction materials, reinforced fiber, bio-energy, bio-bleaching, enzyme, feed, enzymatic hydrolysis (Tsuda*et al.*,1998; Scurlock*et al.*, 2000, Lee *et al.*, 2001; Kobayashi *et al.*, 2004; Vu *et al.*, 2004; Isroi*et al.*, 2011). The fast-growing woody

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plant species can be harvested after 8-20 years, while the harvesting period for bamboo is only 4 years (Nasendi, 1995). The previous study reported that large (*betung*) and yellow bamboos afforded better fiber quality in terms of pulp and paper utilization (Fatriasari and Hermiati, 2008).

The lignin content of the lignocelluloses materials for pulp and paper is undesirable therefore it should be substantially reduced to particular level before utilizing it by using either conventional or non conventional processes Recently, biological pretreatment that included non-conventional approach is more interesting concurrent with the increasing concerns of environmental impacts. In the biological pretreatment of the lignocellulosic materials, white-rot fungi are utilized to split the complex bond of cellulose-lignin by extraction or decomposition of the lignin (Zadrazil *et al*., 1999). These fungi are known micro organisms being able to remove the lignin efficiently from plant cell walls (Haakala *et al*., 2005; Akhtar *et al*., 1998). These fungi secrete the lignolytic enzymes that penetrate into the plant tissues and further perform the degrading action on the lignocellulosic substrate (Messner and Srebtonik,1994; Kirk *et al*.,1980). In metabolizing (e.g. depolimerizing and breaking down) the lignin polymers, a few of the white-rot fungi also unavoidably degrade the carbohydrate polymer in plant tissues (Blanchette, 1991; Istek, 2006). Thorough selection (screening) of white-rot fungi which has high delignification selectivity was an important step to improve the high yield in bio-pulping process.

In general, single culture of white-rot fungi in liquid inoculums is commonly used in the biological pretreatment of lignocellulosic materials. *Cereperiopsissubvermispora* is a well known fungus that has high delignification selectivity in many solid substrates, but this fungus did not grow well on large (*betung*) bamboo. The other fungi such as *Trametesversicolor*, *Phanerochaetechrysosporium*, *Pleurotusostreatus*, *Schizophylumcommmune* have been applied both in single culture inoculums using bamboo as substrate (Fatriasari *et. al*., 2011,2009; Falah *et al.,* 2010; Fitria *et al*., 2012), and in mixed culture inoculums (Fatriasari *al*., 2010), prior to chemical and semi-chemical pulping. The other lignocellulosic substrates such as rice straw (Ermawar *et al*., 2006), bagasse (Fitria *et al*., 2007; Anita *et al*., 2011a, b; Fajriutami *et al*., 2011, 2012), oil palm empty fruit bunch (OEFB) (Risanto *et al*., 2012), aspen wood (Chi *et al*., 2007) were also studied using single culture and mixed culture of three kinds of fungi for ethanol production. In addition combination of white-rot fungi (*P. chrysosporium*) with brown-rot fungi (*Fomitopsispolustris*) had already been conducted to improve microwave performance and enzymatic hydrolysis action too (Fatriasari and Anita, 2012a,b). Investigation regarding the effect of morphological and anatomical characteristics, after single culture inoculums pretreatment, on large (betung) bamboo had already been done (Fatriasari *et al*., 2012). Meanwhile, their possible changes after mixed culture inoculums pretreatment unfortunately have not been reported yet.

In wood and many other micro structure environments, fungi are commonly living and growing in a symbiotic system or in an interaction. Mixed fungi cultures could lead to a higher enzyme production through synergistic interactions, but the final result seems to depend on the combination of the used particular fungi species, the mode of interaction between species, and the micro structure environments or nutritional conditions in the lignocellulosic substrate under colonization (Gutierrez-Correa and Tengerdy, 1997 *in* Chie *et al*., 2007). The previous study employing mixed culture of white-rot fungi pretreatment on large (*betung*) bamboo, which was later pulped using alkali process, showed that the co-culture of two white-rot fungi species produced pulp with better pulp properties, compared to the properties of pulp from corresponding bamboo, which was pretreated with the mixing of three fungi inoculums. The best synergetic interaction was found on co-culturing *P. chrysosporium* and *T. versicolor* inoculums for 45 days. This study was conducted to investigate the possible pattern changes in anatomical and morphological characteristics of large (*betung*) bamboo, attributed to the mixed fungi culture pretreatment.

II. METHODOLOGY

A. Preparation of materials

Fresh, bark-less of 2 year old large (*betung*) bamboo (*Dendrocalamusasper*), originating from Nanggewer, Cibinong was cut to smaller sizesusing a drum chipper and hammer-mill to obtain bamboo chips of about ± 1.6 cm in length. The chips were then stored in a refrigerator to avoid microorganism contamination. Afterwards, they were kept for 24 hours at room temperature, and then sterilized in an autoclave for 45 minutes at 121^{0}C before fungi application.

B. Biological pretreatment

Trametesversicolor, *Phanerochaetechrysosporium* and *Pleurotusostreatus* fungi inoculums were separately cultured on malt extract agar (MEA) *slant* (10.65 grams of MEA in 300 ml aquades) for 7-14 days. Five ml of the *JIS* (Japan Industrial Standard) *broth* medium, which was made by adding 3 g KH_2PO_4, 2 g $MgSO_4.7H_2O$, 25 g glucose, 5 g peptone, and 10 g *malt extract into 1L of aquades*, was injected intoevery14 days by cultured fungi and then scratched by ose. The suspension of fungi was then poured into 95 ml of JIS broth medium and incubated at 27^{0}C for 7-8 days. After incubation, 10 gram of *corn steep liquor* was then poured into 100 ml of fungi inoculums. They were then homogenized by a high speed warring blender twice, each for 20 seconds. The obtained homogenized solution was used as inoculum stock.

The air dry bamboo chips, equivalent to their oven dried weight (ODW) of 250 g, were put into heat-resistant plastic bag. There were four treatment groups namely, (1) mixed inoculum stock of *P. ostreatus* and *T. versicolor* (designated as TVPO); (2) mixed inoculum stock of *P. ostreatus* and *P. chrysosporium* (as POPC); (3) mixed inoculum stock of *P. chrysosporium* and *T. versicolor* (as TVPC); and (4) mixed inoculum stock of *P. ostreatus*, *T. versicolor* and *P. chrysosporium* (as TVPCPO). 25 ml of each liquid inoculum stock was injected into bamboo chips and then those were incubated in room temperature of 29-30^{0}C for 30 and 45 days.After the incubation, part of the samples was treated with maceration process to determine the anatomical characteristics.

C. Fiber characteristics

For examining the characteristics of bamboo fibers, the bamboo samples should be initially macerated.The procedures for maceration were similar to those commonly employed for other lignocellulosic fibers, in accordance with the so-called Schultze method (Sass, 1961). As such, the bamboo sample were heated slowly to 50-60^{0}C in the mixture of concentrated acetic acid and hydrogen peroxide (H_2O_2) at a ratio of 1:1. The heating process took 12-24 hours to ensure the complete separation of fiber-to-fiber (interfiber) bonds or other lignocellulosic tissues into individual cells. The separated fibers were afterwards washed by water, and then were colored by safranin-O. To examine the fiber dimensions, the separated cells were placed on the object glass; ethanol-glycerin was then added, and the cells were evenly spread using a coarse needle before closing the object glass with cover glass. The sample was then ready for microscopic observation to measure the fiber dimensions of both the fungi-pretreated bamboo as well as the untreated bamboo (as control).

Fiber dimensions measured included fiber length, lumen diameter, fiber diameter, and cell-wall thicknesses. The measurement for fiber length was performed 30 times, while for diameters and thicknesses 15 times to obtain their average values. The values of fiber dimensions were then used to calculate their derived values of Runkel ratio, felting power, Muhlstephratio, co-efficient of rigidity, and flexibility ratio. In addition, the macroscopic and microscopic characteristics of the intact bamboo tissues (not macerated) were also observed using optical microscope. Such observation was performed by viewing the cross-sectional characteristics of bamboo sample to obtain the microscopic features of its tissues.

III. RESULT AND DISCUSSION

Fiber dimension and its derived value are important indicators to predict the traits of the resulting pulpand paper. Fiber length of treated bamboo was up to 2 mm (Table 1), and therefore it was categorized as long-fiber and it was judged

as grade-I based on the criteria for Indonesia's woods as raw materials for pulp and paper. Compared withthe untreated bamboo, fiber length of the treated samples tended to decrease. It might be related to the activity ofthe white-rot fungi (WRF) which degraded easily accesible hemicellulose over cellulose to provide nutrient for supporting its growth and methabolism and left the cellulose (Yu *et al*., 2009). The mycelium grew first on the outside and 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, thus fibrillation occurred (Li *et al*., 2010). WRF treatment could intensify the lignin degradation in bamboo through their ligninolytic enzyme activity (Mosai *et al*., 1999; Bjapai *et al*., 2004). This ligninolytic enzyme influenced the changes of anatomical characteristics of bamboo cells. Both the fiber and lumen diameters after WRF pretreatment became wider, while cell wall thickness was thinner. The thin cell-wall will affect positively the flexibility of fibers by providing the broader contact-surface area for inter fiber bonding. The decreasing of cell wall thickness has been predicted, it was caused by the enzyme activity that depolymerize the lignin structure of bamboo cell wall.The lignin fragmentation led to decrease in the lignin contentof the bamboo. The widest and narrowest lumen diameter of bamboo cells occurred following the consecutive pretreatment with POPC for 45 days and with TVPO for 30 days, respectively (Table 1). Co-culture of both POPC and TVPO showed the most intensive activity as indicated by the substantial decrease in bamboo cell-wall thickness. A thick cell wall causes formation of rough and thick paper sheet with high tear strength, but the tensile and folding strengths were predictably low. A thick fiber-wall tends to maintain its rigid and round shape during the sheet formation. This situation can complicate the fibrillation process during the beating/refining actions of lignocellulosic fibers (Casey, 1980). The lower inter fiber bonding between fibers with thick cell wall is caused by two reasons. First, the paper sheet is formed on the weight basis, therefore the number of fibers in the sheet (as retained on the forming wire) is inversely proportional to the density of the cell wall; and second, a thick cell wall hasa smallersurface area per weight unit than thin cell wall. Accordingly, the fibers with thick cell wall explain their small possibility to form perfect inter fiber bonding (Haygreen and Bowyer, 1996).

In general, fungi pretreatment resulted in as light decrease in bamboo fiber length, as compared to that of untreated bamboo/control (Table 1), and therefore were categorized as long-fiber. Further, the fiber length of pretreated bamboo by PCPO for 45 days exhibited the highest value; mean while the TVPC pretreatment for 30 days produced the lowest value in fiber length (Table 1). It means that co-culture of TVPC for 30 days indicatively performed the most intensive delignification activity. A long fiber will enhance its flexibility and provide better fiber-entanglement and greater contact-surface area, and therefore accelerate a better inter fiber bonding formation through hydrogen bond in the paper forming process. Therefore, the longer the fiber, the better the paper properties. The fiber length and fiber derived value such as flexibility ratio affected positively the tear, tensile index and folding endurance (Pasaribu and Tampubolon, 2007). The tear strength is strongly influenced by fiber length and directly related until the fiber length reaches 4-5 mm. A good inter fiber bonding causes a formed paper-sheet with high tear strength and impenetrableto light (Haygreen and Bowyer, 1996). Long fiber also produces the sheet with higher tear strength, as it is related to the formation of the fiber-to-fiber bonding of the wider contact-surface area than short fiber (Syafii and Siregar, 2006). A better inter fiber bonding can be potentially provided by lignocellulosic stuffs with long fiber (Abubakar *et al*., 1995) Long fiber in pulp and paper can improve tear strength of paper (Hubbe and Heitmann, 2007).

White-rot fungi not only produce a whole set of enzymes for lignin degradation but also can act as a transporter for these enzymes into the wood flakes, which further can change the psychological conditions required for enzymatic reaction (Islam *et al*., 2007). *T. versicolor* produces enzymes such as manganese peroxidase (MnP), laccase, hemicellulase, and cellulose (Yang *et al*., 2007). These enzymes use low molecular weight mediator to attack lignin (Perez *et al*., 2002). Lobos *et al*. (2001) and Hossain and Anantharaman (2006) mentioned that *P. ostreatus* and *T. versicolor*

produce 3 types of lignolytic enzymes, i.e. laccase, lignin peroxidase (LiP), and manganese peroxidase. LiP, has the structural difference from that of MnP in the ability to oxidize chemical bonds in lignin. On the other hand, MnP degrades lignin indirectly by providing H_2O_2 for the bio-reaction of lignin with peroxidase (Hossain and Anantharaman, 2006). These enzymes were discovered in *P. chrysosporium* was MnP (Kirk and Chang, 1990). LiP demonstrated the ability to oxidize the lignin bond. In contrast, MnP degrades lignin indirectly by providing H_2O_2 for precursors (Kirk and Chang, 1980; Hossain and Anantharaman, 2006).

There were three possible interactions between the funguses. i.e. (1) antagonistic interaction that produces more rapid exploitation of nutrition, parasitism, (2) other forms of interaction deadlock which are typically no fungal hypae that penetrate into the substrate; and (3) synergistic interaction in which the fungi play a role in the interaction of degrading the same substrate (Boddy, 2000). Therefore, the phenomenon of the decreased fiber length, cell wall thicknesses as well as the increased lumen and fiber diameter might occur where the species of fungus act synergistically/mutualistically.

The requirements of the lingo-cellulosic fiber as raw material for pulp/paper with respect to fiber dimension and its derived values, and the results of bamboo-fiber scoring based on its fiber dimension/derived value are presented in Table 2. The pulp and paper properties are also influenced by fiber derived values. These values/parameters mostly affect the physical properties of the paper sheet. As an example, Runkellratio is theratio ofthe cellwall thickness to lumen diameter, where the value/ratio less than or equalto oneis considered very good forthe paper.

Fibrillationcan be used to improve fiber-to-fiber contact. A high Runkel ratio indicates that fibers are more resistant to external forces (milling, pressing and drying), and they are harder to form into paper sheet. Pretreated bamboo by mixed culture of WRF showed a good fiber derived value, and the pulp quality was judged as grade I based on FAO and LPHH criteria with the total score of 525. There was no significant difference in fiber derived values between treatment combinations (mixed culture) of WRF. It means that the treated bamboo materials will predictably produce pulp/paper with good (satisfactory) properties.

Table 1.Values of bamboo-fiber dimensions

Discription	Mixed inoculum of white-rot fungi species / Incubation days								
	TVPC	POPC	TVPO	TVPCPO	TVPC	POPC	TVPO	TVPCPO	Control[1]
Fiber dimensions	30 days				45 days				
Fiber Length/L (mm)	3.51736	3.87926	3. 83307	3.5844	3.89971	4.10329	3.65865	3.78048	4.693
Grade	1	6	5	2	7	8	3	4	
Fiber Diameter/d (mm)	0.02900	0.031230	0.02928	0.031232	0.02955	0.03072	0.02968	0.029488	0.025
Grade	1	7	2	8	4	6	5	3	
Lumen diameter/e (mm)	0.024528	0.,026368	0.024416	0.026368	0.024896	0.026464	0.025392	0.024896	0.007
Grade	3	6	2	7	4	8	5	4	
cell wall thickness/w (mm)	0.00224	0.002432	0.002432	0.002432	0.,002328	0.002128	0.002128	0.002296	0.009
Grade	7	4	4	4	5	8	8	6	
Score total	12	23	13	21	20	30	21	17	
Rank	7	2	6	3	5	1	3	4	

Remark: 1) Source: Fatriasari and Hermiati, 2008; TVPC stands for the mixed inoculum stock of *P. chrysosporium* and *T. versicolor*; POPC for that of *P. ostreatus* and *P. chrysosporium*; TVPO for that of *P. ostreatus* and *T. versicolor*; and TVPCPO *P. ostreatus*, *T. versicolor* and *P.chrysosporium*

In comparison with the untreated bamboo samples (Fatriasari and Hermiati, 2008), all treatments with fungi caused positive effect in decreasing the Runkell ratio and the coefficient of rigidity, as well as increasing the flexibility ratio. Such occurring phenomena (decrease/increase) were affected by both the thinner cell wall and wider cell diameter, due to fungi action. This result is in line with our previous study on single culture pretreatment of large (*betung*) bamboo (Fatriasari *et al*., 2012). This condition will enable the bamboo pulp to form a paper sheet with high tensile index. Flexibility ratio is the ratio of fiber length to the fiber diameter, whereby this ratio reveals a parabolic-shaped correlation with the paper tensile index and breaking length (Pasaribu and Silitonga, 1974 *in* Utama, 1995). Further, the factors that affect the paper tensile strength are the intensiveness/quality of inter fiber bonding, extents of pulp fibrillation, presence of fines/ degraded fibers, and amount of fillers (Elyani *et al*., 2011). Lignocellulosic fibers with high flexibility produce paper with good breaking length, un-rigid (flexible) fiber properties, and high tensile strength.

Coefficient of rigidity is the ratio between cell wall thickness and fiber diameter. This coefficient was expected to have a negative correlation with tensile strength of paper. Almost all of the pretreated bamboos gave pulp with lower coefficient of rigidity. There is a sharp decline in this coefficient/value compared with that of untreated bamboo (Fatriasari and Hermiati, 2008). Such favorable phenomena could occur on pretreated bamboo with mixed culture of WRF, as it resulted in the thinning of the cell-wall thickness and the widening of fiber diameter. On the other hand, the longer incubation period tended to decline the value of rigidity coefficient. This can be understood as the longer the period, the more intensive the depolymerization of lignin structure, rendering the bamboo fiber less rigid (un-rigid). Un-rigid fibers can provide better fiber-to-fiber bonding, thereby yielding paper sheet with good qualities of internal bond.

The ratio of lumen diameter to fiber diameter can be expressed as felting power/slenderness. The higher the ratio, the better the formation of inter fiber bonding in the paper sheet (Rhamdani, 1994). The fibers of the treated bamboo apparently had loose arrangement, thereby predictably giving paper sheet with lower tear index. A sharp increase in Muhlsteph ratio occurred to all fibers of pretreated bamboo; however the longer incubation time tended to decrease such ratio regardless of kinds/species of fungi. The lower Muhlstep ratio usually leads to the lowering of folding-endurance of pulp/paper sheet (Rhamdani, 1994), and therefore causes unsatisfactory quality of the product. Tear strength of the pulp/paper is closely related to the felting power of the fiber, that is the higher the power the greater the tear strength. Intensive as well as perfect interfiber bonding produces paper sheet with high tear strength and density (Haygreen and Bowyer, 1996). Considering the convenient derived values of treated bamboo fibers with the four co-culture treatments, i.e. TVPC, POPC, TVPO, and TVPCPO (Tables 1 and 3), expectedly this enables the treated bamboo fibers to produce paper sheet with high breaking length up to a particular extent. This is because, based on previous experiment, there is a parabolic-shaped correlation between those derived values and breaking length. Further more, flexibility ratio is the ratio of lumen diameter to fiber diameter, whereby the higher the ratio the bigger the lumen diameter and smaller the fiber diameter. Lignocellulosic fibers with higher flexibility ratio can provide greater breaking length for the paper sheet.

Based on this scoring data of bamboo fibers, bamboo pretreated by POPC for 45 days incubation exhibited the most-convenient fiber dimension and fiber derived values (Tables 1 and 3) with its score reaching 63.

The study reported by Fatriasari *et al.* (2010), showed that TVPC pretreatment for 45 days, prior to the kraft pulping produced the pulp with the best properties. Besides, the co-culture using two kinds of WRF provided pulp with better performance than the mixing of three kinds (species) of fungi inoculums into the bamboo-fiber-containing substrate, although its fiber derived value did not achieve the best score. In this study, inoculum mixing of PC, TV and PO also showed good anatomical properties. It indicated that pulp properties were affected by the employed pulping condition.

Table 2. Requirement of lignocellulosic fiber stuff, with respect to the fiber dimension (fiber length) and the derived values, and raw material for pulp/paper; and results of evaluation (scoring) on the fungi-pretreated bamboos based on their fiber dimensionsand derivedvalues

Discription	Incubation days	Requirement		Mixed culture of white-rot fungi											
				TVPC	Quality Class (Grade)		POPC	Quality Class (Grade)		TVPO	Quality Class (Grade)		TVPCPO	Quality Class (Grade)	
		FAO	LPHH		FAO	LPHH		FAO	LPHH		FAO	LPHH		FAO	LPHH
Length of fiber (mm)	30	>2.00 mm	2.200µ	3.517	I(100)	I(100)	3.879	I(100)	I(100)	3.8331	I(100)	I(100)	3.5844	I(100)	I (100)
	45	>2.00 mm	2.200µ	3.900	I(100)	I(100)	4.103	I(100)	I(100)	3.6587	I(100)	I(100)	3.780485	I(100)	I(100)
Runkell ratio	30	<0.25	0.25	0.188	I(100)	I(100)	0.192	I(100)	I(100)	0.205	I(100)	I(100)	0.189	I(100)	I(100)
	45	<0.25	0.25	0.194	I(100)	I(100)	0.166	I(100)	I(100)	0.173	I(100)	I(100)	0.,193	I(100)	I(100)
Felting Power	30	> 90	90	129.778	I(100)	I(100)	129.494	I(100)	I(100)	129.198	I(100)	I(100)	110.053	I(100)	I(100)
	45	> 90	90	132.495	I(100)	I(100)	129.010	I(100)	I(100)	116.857	I(100)	I(100)	134.832	I(100)	I(100)
Muhls tep ratio	30	>60	60	224.000	III(25)	IV(25)	243.200	III(25)	IV(25)	226.400	III(25)	IV(25)	242.400	III(25)	IV(25)
	45	>60	60	232.800	III(25)	IV(25)	212.800	III(25)	IV(25)	212.800	III(25)	IV(25)	229.600	III(25)	IV(25)
Flexibi lity ratio	30	>0.6	0.6	0.643	I(100)	I(100)	0.641	I(100)	I(100)	0.645	I(100)	I(100)	0.642	I(100)	I(100)
	45	>0.6	0.6	0.640	I(100)	I(100)	0.659	I(100)	I(100)	0.654	I(100)	I(100)	0.640	I(100)	I(100)
Coeff. of Rigidity	30	<0.1	0.1	0.079	I(100)	I(100)	0.080	I(100)	I(100)	0.078	I(100)	I(100)	0.079	I(100)	I(100)
	45	<0.1	0.1	0.080	I(100)	I(100)	0.070	I(100)	I(100)	0.073	I(100)	I(100)	0.000	I(100)	I(100)
Score Total	30				525	525		525	525		525	525		525	525
	45				525	525		525	525		525	525		525	525

Remarks:

1) Runkel ratio =2w/l L=Fiber length
2) Felting power =L/d d=Fiber diameter
3) Flexibility ratio =l/d l=Lumen diameter
4) Coefficient of rigidity =w/d w=Cell wall thicknesses
5) Muhlsteph ratio $= \frac{(d2 - 12 \times 100\%)}{d2}$;

-For the abbreviated codes of TVPC, POPC, TVPO, and TVPCPO, please refer to Table 1
-LPHH= Center for Research and Development on Forestry Engineering and Forest Products Processing, Bogor (Indonesia)

Table 3. Summary of scoring of fiber dimension's derived values for fungi-pretreated bamboo

White rot fungi treatment		Incubation periods (days)	Fiber derived value						
			Runkell ratio	Felting power	Muhlstep ratio	Flexibility ratio	Coeff. of rigidity	Score Total	Rank
Mixed culture	TVPC	30	6	6	7	5	5	29	3
		45	2	7	3	2	4	18	4
	POPC	30	4	5	1	3	8	21	5
		45	8	3	8	8	6	33	1
	TVPO	30	1	4	6	6	4	21	5
		45	7	2	8	7	7	31	2
	TVPCPO	30	5	1	2	4	5	17	3
		45	3	8	6	2	4	23	4

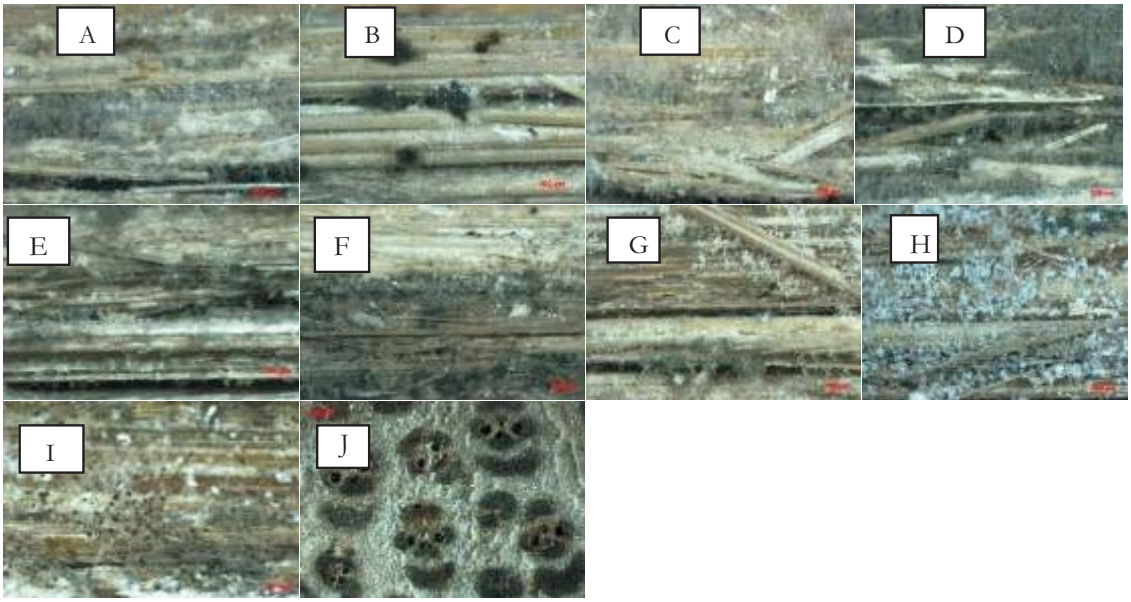

Figure 1. Microscopic image of pretreated bamboo by *P. ostreatus* and *P. chrysosporium* (POPC) 30 days (A), POPC 45 days (B), *P.ostreatus*and *T. versicolor* (POTV) 30 days (C), POTV 45 days (D), *T.versicolor* and *P. chrysosporium*(TVPC) 30 days (E), TVPC 45 days (F), *T.versicolor*, *P. chrysosporium* and *P. ostreatus* (TVPCPO) 30 days (G), TVPCPO 45 days (H), untreated bamboo (I), vascular bundle (J)

The figure below show the macroscopic and microscopic images of bamboo tissues under white-rot fungi treatment. The image indicates that the degradation patterns as occurred were caused by fungi activity in the substrate (A-H).Fiber proportion per mm^2 (Fatriasari *et al.*, 2012) of bamboo tissue was dominated by parenchyma cells (65.18%), followed in decreasing order by fiber cells (31.85%) and metaxylem tissues (1.44%). A high parenchyma cell proportion tended to produce pulp with lower yield. The fungi secreted the hyphae to penetrate into the cell wall via bamboo surface. Macroscopic vascular bundle of untreated bamboo can be seen in images A-J. This bundle has a function as food and water channel. These figures show that the fungi colonized the bamboo chips, degraded their cell walls and detached the fibers. Partial degradation of cell lumen walls was evident. Those studies showed that there seemed to be no substantial effect on bamboo cell walls or modification of cell wall within a relatively short time after inoculation, although the amount of lignin was not significantly removed. SEM

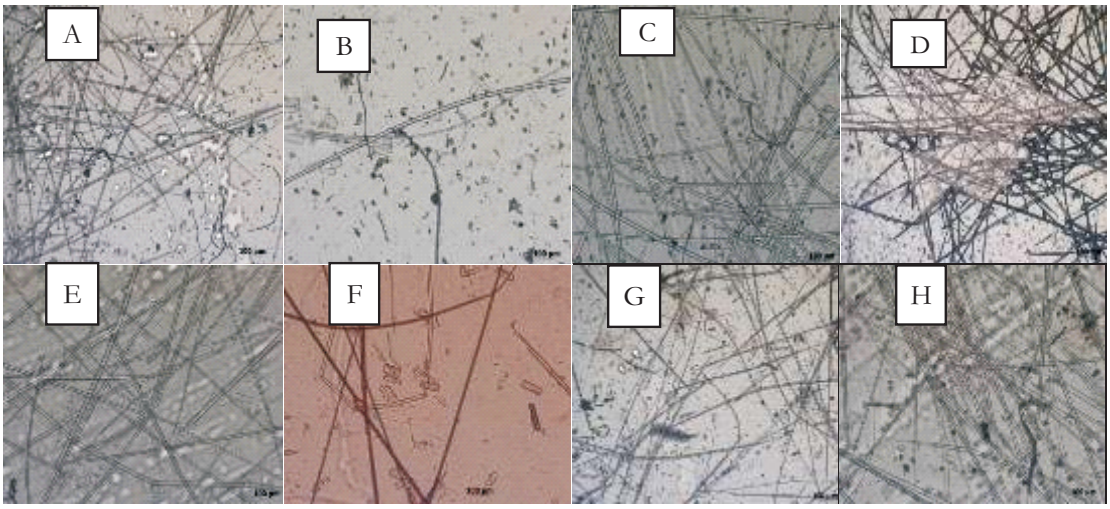

Figure 2. Microscopic image of pretreated bamboo fiber by *P. ostreatus* and *P. chrysosporium* (POPC 30 days (A), POPC 45 days (B), *P. ostreatus* and *T. versicolor* (POTV 30 days (C), POTV 45 days (D)), *T.versicolor* and *P. chrysosporium* (TVPC 30 days (E), TVPC 45 days (F)), *T.versicolor*, *P. chrysosporium* and *P. ostreatus* (TVPCPO 30 days (G), TVPCPO 45 days (H))

(scanning electron microscope) images as reported by Fatriasari *et al.* (2010) showed that the degradation activity of WRF occurred on the substrate. Other studies on the microscopic level of the fungal growth patterns of *P. chrysosporium* and *C. subvermispora* in aspen wood chips showed that *P. chrysosporium* grew well on both across the chip surfaces and throughout the cell wall. Degradation of cell wall possibly occurred. As such, the fungi might sustain the decolonization in all planes of the xylem tissue causing its decay, firstly via the vessels and later on via ray parenchyma. The decay captured the nutrient that was reserved within ray parenchyma and then colonized it to be distributed to the fungal mycelium throughout the sapwood. (Akhtar *et al.*, 1998). Therefore, to clearly identify the changes on the cell walls degradation, further examination using the ultra-structural technique is necessary.

The maceration of mixed-culture-treated bamboo-fiber caused the bundles of fiber to separate through their common cell-wall into single (individual) fibers (Figure 2). The fungi degrading action that caused the physical damage to single bamboo fibers occurred mainly in pretreated bamboo by *T. versicolor* for 45 days (Fatriasari *et al.*, 2012). However on the mixed culture fungi treatment, there seemed to be no significant difference in the effect amongthe mixed culture treatment on the fiber separation.

IV. CONCLUSION

Implementation of mixed culture of various white-rot fungi species enhanced the performance of fiber morphology (i.e. fiber dimensions and their derived value). As such, there was a decrease consecutively in bamboo fiber length, cell wall thicknesses, while with respect to the fiber and its lumen diameter, the changes were on contrary. Compared to the untreated bamboo, all the fiber-dimension-derived values of the fungi-treated materials tended to improve (positively affecting the pulp/paper properties), except for Muhlstephratio. As such, the treated bamboo would indicatively require milder pulping condition, thereby lessening fiber degradation; and hence predictably producing a good quality pulp (grade I) based on FAO and LPHH requirements. The combination of *P. chrysosporium* and *P. ostreatus* treatment for 45 days provided the bamboo with the best fiber dimension and its derived values were most satisfactory. The white-rot fungi grew well on the bamboo substrate, and the corresponding fungi hypae were colonized on the bamboo-surface area followed by the mycelium penetration into the substrate. The evidence of this partial degradation in the fungi-treated bamboo caused by lignin depolymerization (compared to the untreated bamboo) was shown by the related macroscopic images.

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