Kraft and Soda Pulping of White Rot Pretreated Betung Bamboo

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Abstract

This research was conducted to study the effects of pre-treatment with white-rot fungi on pulp properties of betung bamboo. Inoculum stocks of white-rot fungi (25 ml) were injected into polybags contained barkless fresh bamboo chips. Each polybag contained 214.9–286.8 g oven dry weight of chips. Bamboo chips in the polybags were inoculated by *Pleurotus ostreatus* and *Trametes versicolor*. Both of them were then incubated for 30 and 45 days at room temperature. Bamboo chips were cooked using soda and Kraft processes. The cooked bamboo chips were then defiberize using disc refiner for 3 times. Pulp yield, kappa number and degree of freeness of the pulp were then analyzed. The treatment of two white rot fungi, gave different effects on the characteristic of betung bamboo pulp. The effects of fungi treatment on kappa number and degree of freeness can be seen only at samples cooked using kraft process. Bamboo treated with *T. versicolor* incubated for 45 days and cooked using kraft process produced the best pulp quality with high pulp yield.

Key words: betung bamboo, biopulping, degree of freeness, kappa number, pulp yield.

Introduction

Bamboo belongs to the family of grasses and it is the most widely used non wood raw materials or the production of paperboards, papers in Asia and feedstock of textile, food, construction, reinforcing fiber, biomass energy (Tsuda et al. 1998, Scurlock et al. 2000, Lee et al. 2001, Kobayashi et al. 2004, Vu et al. 2004). Bamboo produces cellulose 2-6 times more than pine does (Herliyana et al. 2005). Its biomass increased by 10-30% daily, while wood biomass increased by 2.5% daily. Bamboo can be harvested after 4 years, while we have to wait for 8-20 years to harvest fast growing trees (Herliyana et al. 2005). Based on fibres morphology and physical and chemical properties, betung and yellow bamboos were better raw materials than tali, andong, ampel and black bamboo to be utilized for pulp and paper (Fatriasari & Hermiati 2008).

Kraft pulping is generally preferred to soda pulping when preparing chemical pulp of bamboo (Casey 1979, Vu *et al.* 2004).The kraft process provides satisfactory delignification as well as high yield and viscosity. The main reason is the fiber dimensions and main chemical constituents of bamboo typically bear a close resemblance to those of woods (Vu *et al.* 2004).

The effect of lignin on bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity (Haug 1993, Richard 2000, Zainol

& Abdul-Rahman 2008). The structural complexity of lignin, high molecular weight and insolubility make its degradation is very difficult (Perez et al. 2002). Pretreatment of lignocelluloses materials remove lignin to and hemicellulose can enhance the hydrolysis of cellulose (Sun & Cheng 2002). According to Fatriasari et al. (2007^a), the optimum conditions of betung and yellow bamboo pulp productions by atmospheric soda pulping were 20% of NaOH, fibrillated for 90 min by beater Hollander and then fibrillated once in stone refiner.

The pulp and paper industry utilized mechanical chemical and pulping processes or its combination to produce pulps with desired characteristics. These processes have their own disadvantages. Mechanical processes are electrical energy intensive and produce paper with higher color reversion rate (tendency to turn yellow with time) as a result of high lignin content in raw materials. Chemical pulping involves the use of excessive chemicals to degrade and dissolve lignin and releasing high strength-cellulose fibres from wood cell walls; however this processes are low yield (Akhtar et al. 1998). The Kraft process provides satisfactory delignification as well as high yield and viscosity, but it also needs more bleaching agent due to darker pulp produced (Sjostrom 1981, Siagian et al. 2003, Afrida et al. 2009). The cooking condition advised is at 16% of active alkaline, 22% of sulfidity, 2.5 hours of cooking time at maximum 165°C and liquor to wood ratio of 1:4.5 (Krisdianto et al. 2007). Thus, it is important to apply or combine several methods in order to reduce lignin content in biomass more economically and produce better quality of pulp (Ermawar et al. 2006).

Biopulping is a pretreatment methods for lignocellulosic materials prior to pulping production processes. Most research on white-rot fungi application in pulping process were applied to wood chips (Akhtar et al. 1992 & 1993, Scott et al. 1995 & 1998, Mosai et al.1999, Behrendt et al. 2000, Hunt et al. 2004, Shukla et al. 2004). Biopulping processes were also reported for other materials, e.g. bagasse (Akhtar et al.1998, Ramos et al. 2001, Bjapai et al. 2004), kenaf and corn cob (Akhtar et al. 1999), and rice straw (Akhtar et al.1999, Bjapai et al. 2004). A few studies reported the advantages of biopulping i.e. energy consumption saving, increasing of sheet strength, increasing in acid groups of wood, and reduce environmental pollution (Messner & Srebotnik 1994, Behrendt et al. 2000, Ferraz et al. 2002, Hunt et al. 2004, Idarraga et al. 2005, Islam et al. 2007, Yang et al. 2007, Zhang et al. 2007, Sing et al. 2010, Yadav et al. 2010).

Perez et al. (2002), also reported that biological treatment removed the wood extractives and reduce pitch and toxic effect. However, Akhtar et al. (1992), Leathman et al. (1990), and Yang et al. (2007) showed that the fungi treatment on wood reduce the pulp strength. Setliff et al. 1990 suspected this contradiction was caused by the use of different type of fungi and different process conditions. The biological treatment of wood, prior to chemical pulping has not been investigated to the same extent as biomechanical pulping (Messner & Srebotnik 1994) and biokraft pulping has been investigated for hardwood (Bajpai et al. 2001) and softwood (Messner & Srebotnik 1994, Wolfaardt et al. 2004), although kraft pulping accounts for more than 80% of the world's annual pulp production (Sjostrom 1981, Haroen 2006). Therefore, biopulping is also potential in non wood pulp productions (Akhtar et al. 1998).

White-rot fungi are defined as the microorganisms that most efficiently degrade lignin from wood (Perez *et al.* 2002, Liew *et al.* 2010). White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocelluloses materials (Sun & Cheng 2002, Zhang *et al.* 2007). Beside produces ligninolysis enzymes, it also penetrated the enzymes into material substrate such as wood chips (Messner & Srebotnik 1994). Therefore, the environmentally friendly, effective cost biopulping process is a recent alternative of pulp production.

Fatriasari et al. (2007^b) reported that 45 days of incubation with T. versicolor application by atmospheric soda process resulted in relatively better pulp compared with other fungi appplication. Ermawar et al. (2006) reported that T. versicolor and *P. ostreatus* have better activity in lignin and holocelluloses degradation in rice straw compared with four other species of white rot fungi. The optimum incubation time was 4 weeks, while the lignin contents were insignificantly different in 4 and 6 weeks of incubation. Fitria et al. (2007) also reported that the optimum application of white-rot fungi were 4 weeks of incubation, 15 ml of T. versicolor inoculums with the lignin loss was 22.29% and alphacellulose loss was 3.75%. Therefore, it is interesting and important to know the effect of T. versicolor and P. ostreatus application in kraft and soda pulping of betung bamboo on the pulp yield, kappa number, degree of freeness, and selectivity of delignification.

Materials and Methods

Sample preparation

Fresh, and barkless 2 years old bamboo betung (*Dendrocalamus asper* (Schult.f.)), from Nanggewer, Cibinong was cut using a hammermil to obtain \pm 1,6 cm bamboo chips. The chips were then stored in a refrigerator to avoid microorganisms contamination. They were then kept for 24 hours at room temperature continued by sterilization in an autoclave for 45 minutes at 121 °C before fungi application.

Inoculum stock preparation

The fungi of *T. versicolor* and *P. ostreatus* inoculum cultured on Malt Extract Agar (MEA) Slant (10.65 g MEA was diluted in 300 ml aquadest) for 7-14 days. Five mililiter of the JIS Broth medium was injected in each slants, and the fungi was then scratched by ose. The suspension was then poured into 95 ml of JIS Broth medium (3 g KH₂PO₄, 2 g MgSO₄.7H₂O, 25 g glucose, 5 g pepton, and 10 g malt extract were added into 1000 ml aquadest) and incubated stationery for 7-8 days. A 10 g of *corn steep liquor* was then poured into 100 ml of inoculum. The inoculum was then homogenized by a high speed waring blender twice each for 20 s. The solution obtained was used as inoculum stock.

Preparation of bamboo pulp

The bamboo chips (214.9-286.8 g of oven dried weight) was put into heat resistant plastic bag and injected by 25 ml of *P*. *ostreatus* or *T. versicolor* inoculum stocks. The bamboo chips were then incubated in a room temperature (29-30 °C) for 30 and 45 days. The chips were analysis by SEM after the incubation time was finished.

The incubated bamboo chips were cooked by soda and Kraft process with the following cooking conditions. Soda pulping was carried out using effective alkali of 20%, liquor to wood ratio of 10:1, 3 h time of cooking, 170 °C cooking temperature. After cooking process, the chips was washed to determine residual alkali, followed by 3 times of fibrillation with disk refiner. Kraft process was carried out using effective alkali of 20%, 15% sulfidity, liquor to wood ratio of 5:1, 3 h of cooking time, 170 °C cooking temperature. After cooking, the chips was soaked in cool water for 24 hours to optimalize the residue of cooking solution. The chips was then washed to remove residual alkali, followed by 3 times of pulp defiberation with disk refiner. Excess of water was then removed, and the screened yield of the pulp was determined.

Pulp analysis

The pulp was analyzed for its kappa number (TAPPI T236 cm-85), degree of freeness (TAPPI T227 om-92 1992). Determination of pulp yield according to TAPPI T210 cm-93 by gravimetric measurements. Delignification selectivity (comparison between carbohydrates and lignin in the pulp) was then determined.

Experimental design and statistical analyses

Factorial design was used in this study. There were three factors studied, species of fungi (T. versicolor and P. ostreatus), pulp cooking methods (kraft and soda pulping), and incubation time (30 and 45 days). Data obtained were statistically analyzed by Analysis of Variance (ANOVA). Besides that, each pulp quality was compared to each other using scoring system. The score ranges from 1 (lowest quality) to 4 (higest quality). The scores of each treatment were sum up and were used for ranking the treatments and choosing the best method of treatment. The highest score was considered the best method.

Results and Discussion

Pulp yield

Pulping yield is one of criteria to determine the selectivity of pulping

process. The higher screen yields of pulp, the more selective the pulping process. Because the lignin degradation rate is higher than that of carbohydrate There are several process stages that starting from cooking processes to refining using disk refiner. Each pulping process stages results in pulp yield lost.

Chemical pulp yield in commercial scale commonly is ranging from 40-50% (Akhtar et al.1998, Karlsson et al. 2006). Pulp yield of kraft process without fungi treatment was much lower than that of commercial scale pulping, while pulp vield of soda process without fungi treatment was much lower than pulp yield reported previously (Vu et al. 2004). However, it is still in the range of commercial pulp yield. There were great pulp yield differences between the two chemical pulping processes. Screened yield of soda process was higher than that of kraft process. This might be caused by the differences of chemicals used in both cooking processes as well as chemicals penetration into the bamboo. In kraft process sodium sulfide was also used besides sodium hydroxide, so that there was more lignin loss from the fiber. The chemicals penetration into bamboo chips in kraft process might be more intensive as well. Even though the pulp yield was still in the range of commercial pulping yield, this yield was lower than that reported by Wolfaardt et al. (2004), which were using pine wood with kraft process.

Figure 1 shows soda and kraft pulping yield from white rot fungi pretreated chips of betung bamboo. In kraft process pulp yield of pretreated samples was higher than that of none pretreated, while in soda process it was vice versa



Figure 1 Screened yield of betung bamboo pulp pretreated with white rot fungi (*P. ostreatus* and *T. versicolor*).

According to Perez et al. (2002), in the bio-pulping process, fungi pretreatment will improve chemical penetration and minimize chemical consumption that brought about the improvement of biokraft pulping yield. Our experiment showed that this was not always happened, since pulp yield also depends on the In this case, fungi cooking process. treatment with kraft process increased the screened yield, while fungi treatment with soda process decreased the yield. This is in agreement with the previous result by Perez al. (2002). Thus, et fungi pretreatment has improved penetrations of sodium hydroxide and sodium sulfide into bamboo chips in kraft cooking, while it did not help improving penetration of sodium hydroxide alone in soda cooking. Statistical analysis also shows that species of fungi and interaction between fungi species and cooking process conditions affected pulp yield significantly.

Interestingly, where soda pulp yield resulted from pretreatment with *P*. *ostreatus* was higher than that from

pretreatment with T. versicolor, while kraft pulp yield was vice versa (Fig. 1). Degradation activity of T. versicolor fungus was more intensive. Ermawar et al. (2006) and Fitria et al. (2007) reported that coincide with the loss of lignin, and holocellulose (alpha-cellulose and hemicellulose) was happened. The longer the fungi incubation times, the higher holocellulose loss. The combination between this fungus and kraft process, which involved more severe conditions, caused more yield loss. Also, in kraft process, the longer the incubation time, the higher the pulp yield, while in soda process the effect of incubation time was This shows that degradation of seen. lignin in bamboo was greater at the longer incubation time, and the degraded lignin was further removed by chemicals used in kraft process. Statistical analysis showed that only the fungi kinds and the interaction between the fungi kinds and cooking process conditions influenced pulp yield significantly.

In pretreatment by *P. ostreatus* with kraft pulping, the screened yield was higher than that of control sample not pretreated This phenomenon with the fungus. indicates that there is an improvement condition, since fungi secrete enzyme to facilitate chemical penetration on the substrate, which was suggested by Perez et al. (2002). Basically, sodium hydroxide is used to soften lignin for facilitating the separation. Sodium hydroxide fiber solution can absorb into amorphous and crystalline structure of the cell wall, it caused swelling of the fiber both in diameter cross section and in lumen cell (Roliadi & Fatriasari 2005). Because of white rot is less selective, a part of hemicelluloses and cellulose were also dissolved during the softening process. Thus, it affected the pulp yield. The decrease of pulp yield was observed in samples pretreated with T. versicolor and cooked using soda process.

Kappa number

Kappa number of pulp related to the degree of delignification of the pulp. This number could be used for the comparison of lignin content among treatments. Pulp with good delignification degree will give lower kappa number. Figure 2 shows that in general fungi pretreatment reduce kappa number, except in those treated with P. ostreatus and cooked with soda process. Therefore, it was clear that white rot fungi treatment could degraded lignin in bamboo, thus reduce kappa number of the pulp produced (Mosai et al. 1999, Bjapai et al. 2004). White rot fungi produce ligninolytic enzymes. Τ. versicolor

produces manganese peroxidase (MnP) and laccase, hemicellulase, and cellulase (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 kind of ligninolytics i.e. laccase, lignin peroxidase (LiP), and manganase peroxidase (MnP). Lignin peroxidase (LiP) has the structural difference from manganase peroxidase (MnP) in that it shows the ability to oxidize chemical bonds in lignin. On the other hand, MnP degrade lignin indirectly by providing H₂O₂ for reaction of lignin peroxidase (Hossain & Anantharaman 2006).

This agreed with our research on pretreatment of rice straw (Ermawar et al. 2006) and sugarcane bagasse (Fitria et al. 2007) by the same fungi. However, it was interesting to note that kappa number was higher in pulp treated with P. ostreatus and cooked using soda process than untreated pulp. The possible cause of this strange phenomenon was not yet found. Theoretically, kraft process could produce pulp with lower kappa number than soda process. In the kraft pulping, the presence of hydrogen sulfide ion and sulfide ion accelerate the delignification process, which was caused by attacking β -aryl ether bond (Sjostrom 1981, Fengel & Wegener 1989). However, the kappa number of pulp produced from kraft process was higher than that from soda process if the bamboo was not previously pretreated by white rot fungi.



Figure 2 Kappa number of betung bamboo pulp pretreated with white rot fungi (*P. ostreatus* and *T. versicolor*) for 30 and 45 days.

Figure 2 also shows that the longer the fungi incubation times the lower the kappa number; especially those cooked using kraft process. This was in accordance with the greater ligninolytic activities of the fungi at the longer incubation time. In soda process there was no effect of incubation time of the two fungi on kappa number. Thus, it seems that fungi showed greater effects than did incubation time. However, a reasonable cause of this needs phenomenon further study. Statistical analysis (α =0.05) shows that species of fungi, incubation time and cooking methods significantly affected kappa number of the pulp, and so did the interaction between species of fungi and cooking methods as well as interaction between incubation time and cooking methods.

Kappa number of betung bamboo pulp without fungi pretreatment was higher than that of *Bambusa procera* pulp as reported by Vu *et al.* (2004). They reported that kappa number of the pulp resulted from soda process using 20% EA (effective alkali) was 33.1, while kappa number of unbleached kraft pulp was ranging from 18 to 30 (Bhargava 1987, *in* Vu *et al.* 2004).

Degree of freeness

Fiber modification (i.e. any changes in the surface and development of fiber) as a result of milling is measured by degree of freeness. Freeness shows a flow of releasing water carried by the pulp suspension, or in other word, shows the ability of the pulp to hold the water. A common measurement of freeness is by using Canadian Standard Freeness (CSF) (Tappi T 227 om-92 1992). Degree of pulp freeness is measured by volume of water which trapped in a side-patch of freeness tester. The more the water flow into the side-patch, the higher the freeness; or the lower the ability of pulp to hold the water flow (Wistara 2000).



Figure 3 Freeness of betung bamboo pulp pretreated with white-rot fungi (*P. ostreatus* and *T. versicolor*) for 30 and 45 days.

The degree of freeness of bamboo pulp treated with P. ostreatus and T. versicolor is shown in Figure 3. In the pulp cooked using kraft process, the freeness decreased in line with the incubation time of T. versicolor treatment. However, compared with the control, only the 45 days treatment significantly incubation decreased the degree of freeness (42.79%). In contrast, the degree of freeness increased in line with the incubation time of P. ostreatus treatment. These results may be as a result of the variability of fiber type that arranged the pulp sheet. If pulp contained high amount of fines then the pulp sheet would become more assembled (Wistara 2000)

The result suggested that there was inconsistency of the effect of incubation time treatment on the degree of freeness. Regardless the inconsistency of the incubation time effect, the fungi treatment decreased the degree of freeness by 34.73% with *P. ostreatus* treatment and/or otherwise increased the degree of freeness

by 29.47% and 26.65% with the treatment of *P. ostreatus* and *T. versicolor* respectively. On the other hand, in pulp resulted from soda process, compare with the control, the treatment of fungi was slightly (but not significantly) decreased the degree of freeness.

Physically, fungi treatment with Т. versicolor has softened the bamboo chips. However, physical modification seems did not give any effects on the degree of freeness. Statistical analysis (α =0.05) shows that only cooking process had significant effect on the degree of freeness. The degree of freeness of bamboo pulp without fungi treatment and cooked using Kraft process was lower than the one produced using soda process. These may be a result of greater penetration of kraft chemical compounds (iNaOH and Na₂S) into the cell wall and delignified the lignin. The result also suggested that fibrillation process, instead of the fungi treatment, was more likely affect the degree of freeness.



Figure 4 Delignification selectivity of betung bamboo pulp pretreated by white-rot fungi (*P. ostreatus* and *T. versicolor*) for 30 and 45 days.

Selectivity of delignification

delignification Selectivity of is а measurement of pulping process which involves effectiveness fungi performance. High value of selectivity means that fungi selectively attack lignin to give a high content of carbohydrate and low content of lignin in pulp. White-rot fungi are expected to give real the selectivity. contribution to gain Statistical analysis (α =0.05) shows that all factors have independently significant effect on the delignification selectivity. There were significant effects due to interaction between fungi and cooking process, and also between cooking process and time of incubation.

The delignification selectivity of both fungi to bamboo pulp is shown in Figure 4. Generally, fungi treatment with *T*. *versicolor* and cooked using Kraft process showed the best performance compare to that resulted from the treatment with *P*. *ostreatus*. In kraft process, both fungi treatment have significantly increased the selectivity. Meanwhile, in soda process, only *T. versicolor* has significantly increased the selectivity. The result also shows that the longer the time of incubation the higher the selectivity.

SEM (Scanning Electron Microscopy)

Figures 5 show bamboo with fungi pretreatment compared to bamboo without fungi pretreatment. It can be showed clearly that white rot fungi activity caused decay on bamboo cell wall. But, the decay rate among each white rot fungi kind could not be determined clearly. For each fungi kinds, the longer incubation time, the greater damage rate of bamboo.Further examination at the level of ultra structure fiber is necessary to observe any changes of the cell walls due to penetration of fungi activity. The degradation of cell wall was possible and the mechanism of this process stated by Akhar et al.(1998). The fungi might be colonized the xylem in all vessels and planes. first via ray parenchyma. The decay captured the nutrient that was reserved within ray and parenchyma then colonize the parenchyma to distribute the fungal mycelium throughout the sapwood.



Figure 5 SEM image of bamboo (PO 30 days (A),PO 45 days (B), TV 30 days (C), TV 45 days (D), and control (E)).

Scoring value

Scoring value of pulp characteristic as a result of Kraft and soda cooking is presented in Table 1 and 2. For every best value in each parameter, the scoring value is 4. The result shows that treatment with

T. versicolor for 45 days incubation has significantly affected the betung bamboo pulp characteristics (shows by a large difference of value compared with the value of other treatment combinations).

Treatments	Incubation time (days)	Kappa Number	Pulp Yield (%)	Degree of Freeness (ml)	Delignification Selectivity (%)	Score	Rank
Dlauratus	30	$54.8^{1}{\pm}11.84$	$30.60^1 \pm 1.06$	$347^3 \pm 6.98$	$13.59^{1} \pm 0.41$	6	4
ostreatus	45	$33.8^3 \pm 0.22$	$35.75^3 \pm 0.94$	$688^2 \pm 6.98$	$22.88^3 \pm 1.70$	11	2
Tramatas	30	$49.4^2 \pm 5.95$	$32.54^2 \pm 0.45$	$673^1 \pm 9.43$	$14.74^{2} \pm 1.44$	7	3
versicolor	45	$27.2^4 \pm 1.96$	$40.22^4 \pm 1.47$	$304^4 \pm 12.62$	$25.03^{4} \pm 3.53$	16	1

Table 1 Kraft pulp properties of betung bamboo with fungi pretreatment

Treatments	Incubation time (days)	Kappa Number	Pulp Yield (%)	Degree of Freeness (ml)	Delignification Selectivity (%)	Score	Rank
Pleurotus ostreatus	30	$97.88^2 \pm 3.97$	$41.37^{4} \pm 1.57$	$734.17^2 \pm 3.54$	$6.83^2 \pm 0.30$	10	2
	45	$99.50^1 \pm 5.26$	$41.28^3 \pm 3.41$	$733.13^3 \pm 3.65$	$6.74^{1} \pm 0.25$	8	4
Trametes versicolor	30	$48.64^3 \pm 20.21$	$30.42^{2} \pm 7.18$	$735.00^{1} \pm 2.36$	$17.35^3 \pm 8.04$	9	3
	45	$44.37^4 \pm 2.49$	$28.2^{1}\pm4.22$	$731.88^4 \pm 0.00$	$17.72^{4}\pm 6.51$	13	1

Table 2 Soda pulp properties of betung bamboo with fungi pretreatment

Conclusions

The treatment of white rot fungi, i.e. *P.* ostreatus and *T. versicolor*, gave different effects on the characteristic of betung bamboo pulp. The effects of fungi treatment on kappa number and degree of freeness can be seen only at samples cooked using Kraft process. Incubation time did not affect pulp yield of bamboo treated with both fungi, but it affected kappa number and degree of freeness of bamboo pulp cooked using Kraft process. Bamboo treated with *T. versicolor* incubated for 45 days and cooked using kraft process produced the best pulp quality with high pulp yield.

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