Effect of Corn Steep Liquor on Bamboo Biochemical Pulping Using *Phanerochaete chrysosporium*

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Abstract

The effect of corn steep liquor (CSL) loading on white rot fungi inoculums in the biochemical pulping of betung bamboo was investigated. The best condition of the pretreatment was also determined. There were two conditions of CSL amount used i.e. 1% (v/w) and 10 % (v/w) of CSL used in 100 ml of inoculums. In short, fresh bamboo chips without bark was incubated with *Phanaerochaete chrysosporium* inoculum stocks for 30 and 45 days at room temperature and followed by Kraft and Soda pulpings. SEM images of pretreated chips were taken after incubation. The pulp yield, Kappa number, freeness, delignification selectivity, and brightness were analyzed. The more CSL amount added with Kraft process gave better results on pulp yield. Increasing incubation time increased pulp yields and decreased the Kappa numbers. On the other hand, freeness and brightness of pulp increased especially for Kraft process. The SEM images showed that there were cell walls degradation activities. The best properties of pulp were obtained by the Kraft process for the treatment of 10% CSL with 30 days of incubation time.

Key words: betung bamboo, biochemical pulping, corn steep liquor, *Phanaerochaete chrysosporium*

Introduction

Bamboo is one of non-wood raw materials that have been widely used for pulp and paper production in Asia (Atchison 1998, Lee *et al*. 2001). Due to the high strength of bamboo pulps, these pulps can be used for more versatile paper products than the majority of other non-wood pulps (Vu *et al*. 2004). *Betung* and *kuning* (yellow) bamboo have better degree of suitability as pulp raw materials compared to other bamboos, such as *andong*, *ampel* and *hitam* bamboos based on analysis of fiber morphology and physicochemical properties (Fatriasari & Hermiati 2008).

Bamboo pulp can be processed chemically, such as using Kraft and soda methods. For bamboo, Kraft pulping is generally preferred to soda pulping when preparing chemical pulp. The Kraft pulping provides satisfactory delignification as well as high yield and viscosity. However, environmentally friendly degradation of lignocellulosic materials i.e. using biological treatment is becoming more essential at the moment (Akhtar *et al*. 1998, Messner & Srebotnik 1994, Reid 1998, Akhtar *et al*. 1993, Messner *et al*. 1998).

Bio-Kraft pulping, which uses fungal pretreatment of wood chips prior to Kraft pulping, is based on white rot fungi degrading and modifying the lignin (Hakalaa *et al*. 2005). White-rot fungi are classified into basidiomycetes and well-

known as the most effective biological treatment for lignocellulosic materials to remove lignin from plant cell (Hakalaa *et al*. 2005, Akhtar *et al*. 1998). However, many of these fungi have the ability not only depolymerize and metabolize lignin but also to degrade cellulose and hemicelluloses (Blanchete 1991). They are not only produce lignin degrading enzyme but also penetrate into substrate (e.g. wood chips) to distribute the enzyme (Kirk *et al*. 1980).

Research on utilization of white-rot fungi in pulping have been focused on wood materials (Akhtar 1998, Mosai *et al*. 1999, Behrendt *et al*. 2000, Hunt *et al*. 2004, Shukla *et al*. 2004). Biopulping for non-wood materials, such as bagasse (Ramos *et al*. 2001), kenaf, rice straw, corn stem and wheat, bamboo (Fitria *et al*. 2006, Fatriasari *et al*. 2007, 2009, 2010) have also been reported. The benefits of biopulping, such as energy saving, reducing the amount of cooking chemicals, increase the cooking capacity or enable extended cooking that resulting in lower consumption of chemicals in bleaching, improvement of various paper strength properties and acid group of wood as well as reducing environmental pollution, were reported in Akhtar *et al*. (1993), Messner and Srebotnik (1994), Messner *et al*. (1998), Hunt *et al*. (2004), Ferraz *et al*. (2002), Sun and Cheng (2002), Behrendt *et al*. (2000). However, some studies reported that treatment of fungi could reduce the pulp strength (Akhtar *et al*.1992, Leathman *et al*.1990). Those differences suggested that characteristic of the resulted pulp was influenced by type of fungi and treatment conditions (Setlife *et al*. 1990).

Biological treatment followed by chemical pulping (biochemical) has resulted in different effect compared to the one with biomechanical. Besides, investigation of biopulping using Kraft was applied for only hardwood materials (Wolfaardt *et al*. 2004).

Selectivity of white-rot fungi regarding lignin degradation depends on the lignocellulose spesies (Hakala *et al*. 2005), cultivation time, and other factors (Hatakka & Hammel 2010). White-rot fungi i.e. *C. subvermisphora*, *D. squalens*, *P. chrysosporium*, and *P. radiata* were included selective decay, whereas *T. versicolor* and *F. fomentarius* could categorized as non-selective decay. In selective decay, lignin and hemicellulose fraction are selective degraded while the cellulose fraction is unaffected. But, in non-selective degradation, all fraction of lignocellulose are degraded (Blanchette 1995, Hatakka 2001). This fungus has high growth rate, rapid metabolism of lignin, high optimum temperature and, low phenol oxidase activity (Leatham & Kirk 1982). Bar-Lev *et al*. (1982) reported that treatment of a coarse mechanical pulp with *P. chrysosporium* decreased the energy required for further fiberization and increased paper strength properties.

There are more than 30 factors affected in biopulping such as species and strain of fungi, type and quantity of inoculums, type of wood, amount and/or size of wood chips, environmental factors, additional nutrition and sterilization of wood chips (Akhtar *et al*. 1997a, 1997b). Corn steep liquor (CSL) is a major by product of corn starch processing. It is inexpensive source of nitrogen, vitamins, amino acids and other nutrients in many fermentations and readily available nutrient source. The nutrients in inoculums stimulate initial fungal growth and establishment in the chips. CSL has been shown to reduce the amount of fungal inoculums required for biopulping (Akhtar *et al*. 1997c). However, CSL is imported and relatively expensive in Indonesia. The previously

study by Akhtar *et al*. (1997c) reported that the addition of 0.5% sterilized CSL saved 28-29% energy and improved tear index to 21-22%.

The study goal was to investigate the effect of corn steep liquor amount in the inoculums stocks of white rot fungi *P. chrysosporium* in soda and Kraft pulping of betung bamboo. The most appropriate condition of biopulping was determined, as well.

Materials and Methods

Materials preparation

Fresh bark-free of 2 years old betung bamboo (*Dendrocalamus asper*) from Nanggewer, Cibinong was cut using a Hammermill to obtain ± 1.6 cm in length of bamboo chip. The chips were stored in a refrigerator to avoid from microorganism contamination. They were kept for 24 h at room temperature continued by sterilization in an autoclave for 45 min at 121 ^{o}C before fungi application.

Inoculum stock preparation

Inoculums of *P. chrysosporium* were cultured on Malt Extract Agar (MEA) *Slant* (10.65 g MEA was diluted in 300 mL aquadest) for 7-14 days. Five ml of the *JIS* broth medium was injected into each slant where the fungi were scratched by ose. The 5 ml of fungi suspension was poured into 95 ml of JIS broth medium (3 g KH_2PO_4, 2 g $MgSO_4.7H_2O$, 25 g glucose, 5 g pepton, and 10 g malt extract were added into 1 l of destilled water) and incubated stationery for 7-8 days. Additional 10 g of corn steep liquor was poured into 100 ml of inoculums. The inoculums were homogenized by a high speed Waring blender twice (each for 20 s). The homogenized solution was used as inoculums stock.

Methods of inoculation

The 250 g of oven dried weight (ODW) bamboo chips was put into heat resistant plastic bag and autoclaved for 45 min at 121 ^{o}C. The chips were cooled at room temperature and injected by 25 ml (10%) of *P. chrysosporium* inoculum stocks. The bamboo chips were incubated in a room temperature (29-30 ^{o}C) for 30-45 days. The chips were analysis by SEM after the incubation time was finished.

Pulp production

Bamboo chips were incubated with *P. chrysosporium* for 30-45 days and cooked by Soda process under following condition: 20% of NaOH to ODW of bamboo, liquor to wood ratio 10:1; 3 h of pulping time, 170 ^{o}C of pulping temperature. After pulping process, the chips were washed and followed by 3 times of fibrillation with disk refiner.

Bamboo chips were incubated with *P. chrysosporium* for 30-45 days and cooked by Kraft process under following condition: 20% of active alkali, 15% of sulfidity to ODW of bamboo, liquor to wood ratio 5:1, 3 h of maximum temperature pulping time, 170 ^{o}C pulping temperature. After pulping process, the chips were soaked in cooking solution for 24 h to optimize the residue of cooking solution.

Pulp analysis

Total yield of the fibrillated pulp was determined using gravimetric measurements (TAPPI T210 cm-93). Kappa number and freeness were analyzed in accordance to TAPPI 236 cm-85 and TAPPI T227 cm-85 respectively. Selectivity delignification (comparison between carbohydrates and residual lignin in the pulp) and brightness were analyzed in accordance to SNI 14-4733-1998 and

SNI 14-0696-1998. The pulp quality produced by the two cooking processes with two concentrations of CSL and two periods of fungi incubation were compared to obtain the best methods of betung bamboo biopulping.

Results and Discussion

Pulp yield

Figure 1 showed pulp yield of betung bamboo pretreated with *P. chrysosporium* white rot fungi for 30 and 45 days. The CSL amount and cooking process affected to the pulp yield obtained while the incubation time showed different effect to the pulp yield. Treatment of 10% CSL resulted in the higher pulp yield produced (34.30-44.65%) than the one with 1% CSL (28.93-43.44%). The highest pulp yield produced was the one that treated with 10% CSL, incubated for 30 days and cooked by Kraft.

In general, the Kraft process also resulted in higher pulp yield (36.95-44.65%) than the soda process, which the resulted pulp yield was about 28.93 to 35.68%. The longer incubation time tends to increase the yield both for Soda and Kraft process, except in 45 days incubation of 10% CSL by Kraft. However, the yield decreased by 5% of the 30 days incubation (Figure 1).

Average bamboo pulp yield pretreated by *P. chysosporium* was lower than that on the wood based commercial-scale chemical pulp (40-55%) (Karlsson *et al*. 2001). This condition might be happened because of two reasons. First, the possibility of high proportion of parenchyma cells within bamboo, which the cells were easily degraded. Second, the hemicelluloses content reduced by approximately 40%, as compared to 20% of the lignin, in the extraction stage of cooking. The hemi-cellulose loss is caused by dissolution of low-molecular-weight carbohydrates, removal of acid groups, and degradation or peeling reaction (Smook 1992).

In the Soda process, there was no cleavage on β aryl ether bond position (Fengel & Wegener 1989, Sjostrom 1995), which β aryl ether bond was a dominant bond in the lignin structure (Butcher 2003, Hon 1996). This condition caused the lignin fragmentation activity lower than the one in the Kraft process. In the Kraft process, the presence of hydrogen sulfide ion and sulfide ion accelerate the delignification process, which was caused by attacking β aryl ether bond and preserving pulp yield (Sjostrom 1995, Fengel & Wegener 1989). The accelerating of delignification has caused more degradation of lignin compared to degradation of its glycosidic bond. In addition, hydrosulfide (HS^-) and sulfide ions, which are additive agents in Kraft, can maintain more cellulose compared to NaOH in Soda process. These ions prevent decreasing of carbohydrate because of stabilization at the end group of cellulose chain.

P. chrysosporium rapidly colonizes chips and use wood sugars and other easily accessible nutrient (Akhtar *et al*. 1998). CSL provided inexpensive in USA and commercial nutrients source in inoculums to stimulate initial fungal growth and establishment in the chips (Akhtar *et al*. 1997a). Similarly, the CSL promoted better growth of fungi which produced ligninolytic enzymes to degrade lignin in the bamboo chips and resulted in higher yield. Fungal treatment in biopulping process will improve chemical penetration and minimize the use of chemicals and on biokraft pulping indicates an improvement in yield (Perez *et al*. 2002). Basically, the use of NaOH as cooking solution serves to soften the lignin and promote separation of fibers.

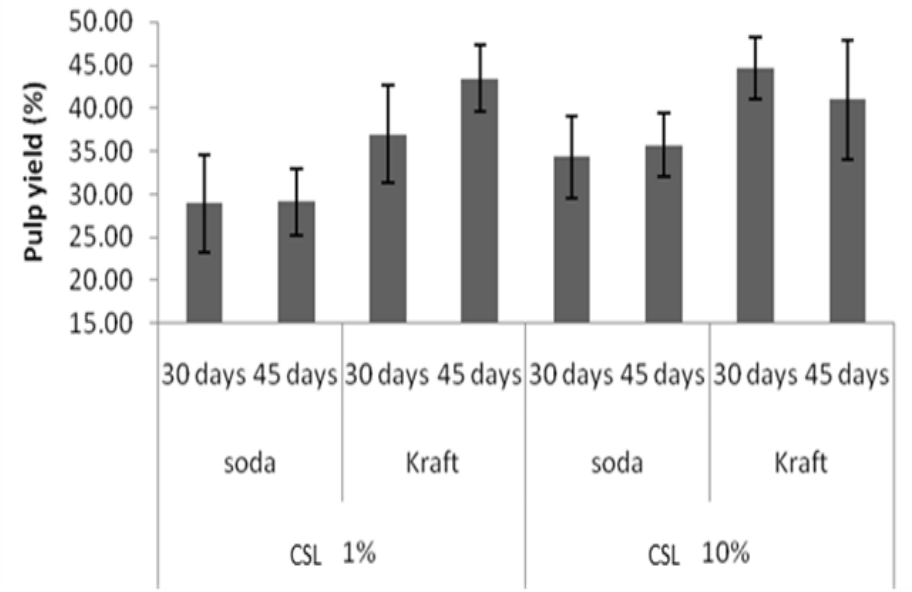

Figure 1 Yield of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi.

NaOH solution can be absorbed into the amorphous and crystalline structures in the cell wall, which leads to the development (enlargement) of cross-sectional diameter of the fiber and fiber lumen and wall thinning. However, combination of pulping process and the activity of white-rot fungi might not interact properly in the process of softening of lignin, which caused some holocellulose dissolved and contributed to the resulting effect on yield. The enzyme secreted by this fungus had less effect on the pulp yield than the pulping solution. White-rot fungi are very selective to lignin for a certain period of time but it also attacks carbohydrates for a prolonged period (Messner *et al*. 1998). In our case, that might be happened when pulp was incubated for 45 days with 10% of CSL in inoculums and cooked by Kraft process. The result showed that more lignin and cellulose were lost due to this combination of treatment compared to that one with 30 days of incubation (Figure 1).

Kappa number

Kappa number shows the residual lignin content of pulp. The numbers can serves as a tool to compare the lignin content among the treatments. Good manufacturing in pulping required a low Kappa number. Figure 2 showed Kappa number of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi for 30 and 45 days. It showed that the lowest Kappa number of pulp was obtained when 1% CSL amount was added in inoculums followed by Kraft cooking (9.15-9.29). The Kraft process resulted in lower (thus better) Kappa number, both for 1% and 10% CSL amount in inoculums (9.15-49.35). This was a 91% reduction compared to the control Kraft pulp (untreated Kraft pulp) (Figure2). While the longer incubation time tends to slightly decrease the Kappa number of pulp with the same treatment. This might be caused by the more intensive attack of fungi into the structure of lignin. The lignin fragmentation leads to the decrease of lignin content.

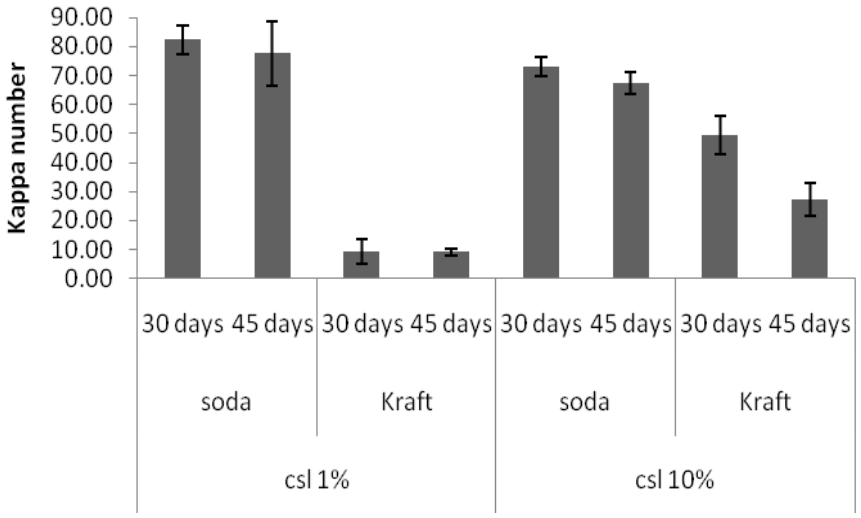

Figure 2 Kappa number of betung bamboo pulp pretreated with *P.chrysosporium* white rot fungi.

Essentially, the swollen lignin in the chips is chemically split into fragments by hydroxyl (OH^-) and hydrosulfide (SH^-) ions present in the pulping liquor. The lignin fragments are then dissolved as phenolate or carboxylate ions (Smook 1992). Kappa number of Kraft process lower than that of Soda process was caused by the attack of hydrogen sulfide nucleofil (Nu) ions into quinone methide (QM) intermediate and formed tyran structure. After that, there was cleavage β aryl ether bond in lignin structure simultaneously and formed styrene type at 170 ^oC with releasing sulfide ion. The presence of hydrogen sulfide ion and sulfide ion accelerate the delignification process in Kraft pulping.

In soda pulping there was less cleavage in β aryl ether bond both for phenolic and non phenolic lignin type. This bond is the most dominant bond in the lignin structure. Besides that hydrosulfide ion is believed to reduce condensation reactions by blocking reactive groups (e.g., hydroxyl in benzyl alcohols) (Smook 1992). Finally, the Kappa number of Soda pulping would be higher than Kraft's.

The Kappa number with 10% CSL in inoculums was higher than that in 1% CSL. White-rot fungi are well known as the most attractive agents for the biological removal of residual lignin from Kraft pulp and are unique among most microorganisms in their capacity to depolymerize and metabolize lignin. In addition, pretreatment improved the penetration of the pulping chemicals to the raw material, thus, enhancing delignification reactions (Oriaran *et al*. 1990).

CSL contains readily available carbohydrates which suitable for microorganism such as fungi to grow. CSL is also a viscous concentrate of corn soluble, rich in proteins and peptides (20–25%), amino acids, lactic acid (7–9%), minerals, vitamins and other growth stimulants, with pH approximately 4. It contains 50–60% solids. Approximately 90% of the nitrogen present in CSL is amino nitrogen, less than 95% of peptides and more than 5% of free amino acids.

The total nitrogen content of CSL, which varies from batch to batch, is 3–5% (Schroeder 1997 *in* Humar & Pohleven 2005). Fungi, like other organisms, require substantial amounts of nitrogen for synthesis of proteins and other cell constituents (Zabel & Morrell 1992, *in* Humar & Pohleven 2005). A further study should be conducted to learn the sharply decreasing of Kappa number in treatment by CSL 1% using Kraft process.

Freeness

Freeness shows the rate at which the pulp suspension solution was carrying off water or, in other words, the ability to hold water. Degree of freeness is generally determined with the Canadian Standard Freeness (CSF) (Ates *et al*. 2008).

Pulp freeness of betung bamboo pretreated with *P. chrysosporium* represented in Figure 3. This figure showed that the incubation time and cooking process effected to the degree of freeness. Results of the longer incubation time (45 days) to the freeness were unexpectedly higher (738.3-742.5 ml) than 30 days of incubation (733.1-737.5 ml). While Kraft pulping yielded in lower freeness than soda process, with the lowest freeness was obtained at 733.1 ml by Kraft process with 30 days of incubation and 10% CSL amount. The CSL amount used showed less effect on the freeness degree obtained.

The lower freeness of Kraft pulp might be caused by the lower lignin content in cell wall as showed by the Kappa number of Kraft process. The presence of hydrogen sulfide ion and sulfide ion accelerate the delignification process in Kraft pulping.. The result also suggested that further examination of the significant increased of Kraft pulp freeness that in line with incubation period and 10% CSL was necessary. In addition, the type of fiber constituent pretreated by fungi affected the freeness value and the more fines in fiber would be given the high freeness value.

In comparison to previous study (Fatriasari *et al*. 2010), the pulp obtained was coarser than the control pulp (532 ml CSF with the Kraft process). In present study, the freeness values obtained at different incubation periods were similar, or in other words, a longer incubation period did not relate positively to the freeness of the resulting fibers. The same results of pulp freeness was reported by Ates *et al*. 2008, where the fungi treatment (*C. subvesmispora* and *P. subserialis*) with Kraft-AQ and soda-AQ processes affected the increasing of pulp freeness.

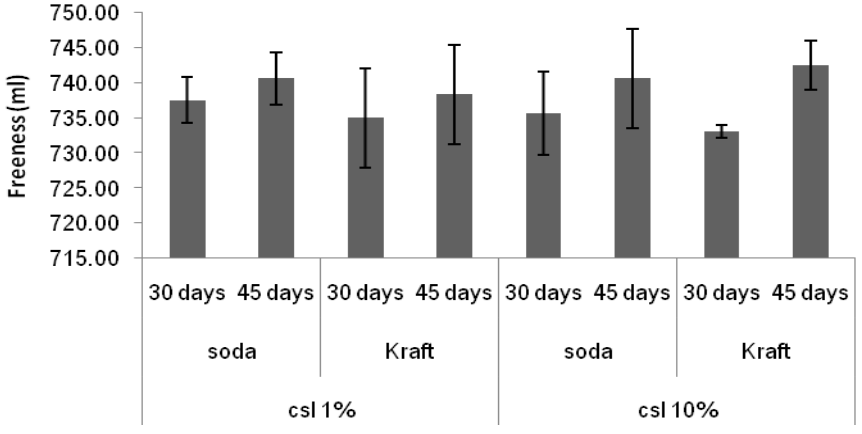

Figure 3 Freeness of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi.

Therefore, to improve freeness value, further examination on fibrillation processes, such as increasing the frequency, are necessary.

Delignification selectivity

Delignification selectivity is a measurement of the effectiveness of the pulping process. A higher selectivity value indicates a more intensive lignin degradation activity compared to the rate of carbohydrate degradation in pulping process. Figure 4 showed delignification selectivity of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi for 30 and 45 days. Generally, the combination of treatments gave low delignification selectivity except for the 1% CSL amount using Kraft process (Figure 4). There was a sharp increasing of delignification selectivity in this treatment. The result suggested that there was more activity on lignin degradation compared to the holocellulose degradation.

In Kraft process, a cleavage of β aryl ether bond in lignin structure happened because of the presence of hydrogen sulfide ion and sulfide ion (Sjostrom 1995, Fengel & Wegener 1989). This condition leads to preserve pulp yield and degrade lignin intensively. The CSL promoted better growth of fungi which produced ligninolytic enzymes to degrade lignin in the bamboo chips and resulted in higher yield obtained. However, there was a possibility that the excess amount of nutrients in the inoculums caused fungi to grow more by consuming these nutrients instead of degrading lignin in the substrate. This condition might be caused more intensive lignin degradation activity in *P. chrysosporium* with 1% CSL using Kraft pulping. Increasing the incubation time for all treatments has proven a positive effect on increasing the value of delignification selectivity although each treatment has a different sensitivity. In line with increasing of incubation time, the lignin degradation activity was more intensive because of deep penetration of fungi into chips.

Brightness

Brightness describes the whiteness of pulp or paper, with a scale of 0% (absolute black) to 100% (relative to Magnesium oxide (MgO) standard, which has the absolute brightness of 96%) by the reflection of light blue (457 nm) of the paper. Unbleached Kraft pulp has an average degree of pulp brightness of 20% (Bierman 1996, Smook 1992).

Pulp brightness of betung bamboo pretreated with *P. chrysosporium* white rot fungi showed in Figure 5. The result showed that pulp brightness of Kraft process both on CSL 1 % and 10% increased sharply compared to soda's process and their control. It was understandable because in Kraft process delignification activity is accelerated by hydrosulfide and sulfide ions that more selective to lignin degradation. Fungi treatment also facilitates the removal of lignin in cell wall by enzyme secretion, thus the lignin content decreased. The study of Ates *et al*. (2008b), Mosai *et al*. (1999) and Copur and Tozluoglu (2007) reported that fungal pretreatment using *C. subvermispora* prior to Kraft pulping had a positive effect on bleaching characteristics of the resulting pulp. Approximate brightness range of unbleached Kraft is 15-30 ISO (Smook 1992). However, the result of present study (Fig. 5) showed that brightness of untreated Kraft pulp (control) was lesser (i.e. less than 10) than the reported range (15-30).

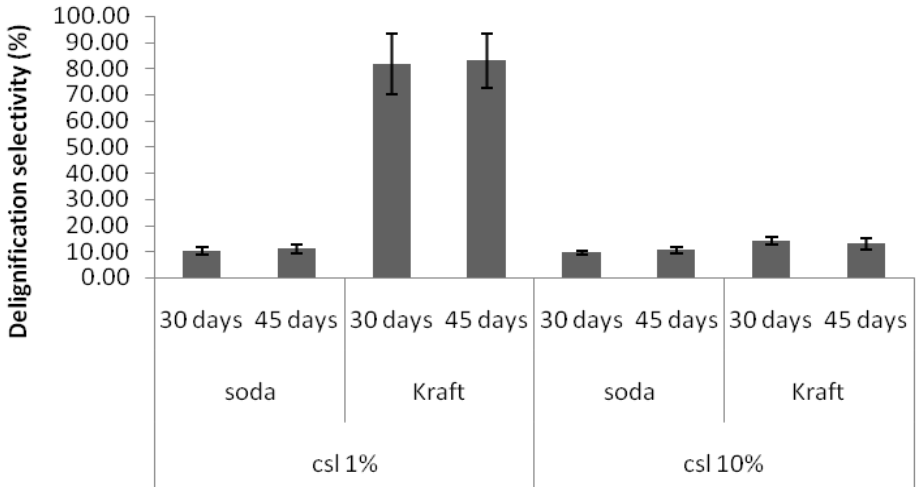

Figure 4 Delignification selectivity of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi.

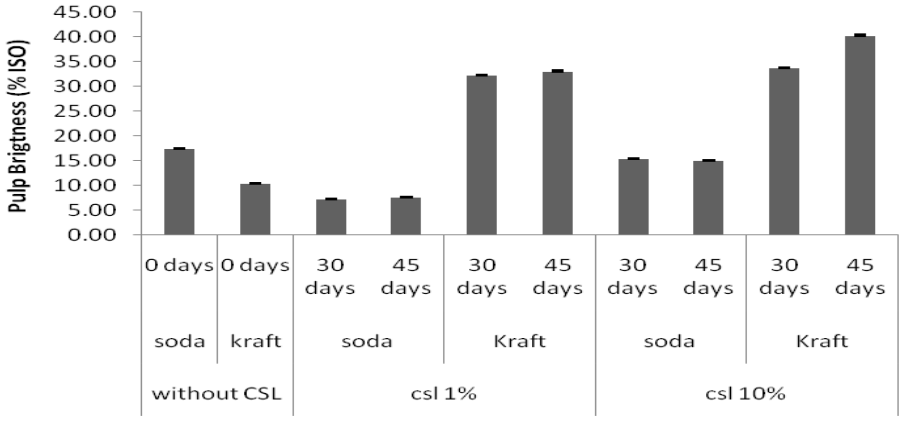

Figure 5 Brightness of betung bamboo pulp pretreated with *P. chrysosporium* white rot fungi.

In addition, there was a tendency that the longer incubation period (45 days) increases the pulp brightness. The same pattern happened in the excess amount of CSL (10%). This might be caused by intensive activity of lignin degradation.

SEM (Scanning Electron Microscopy) observation

Figure 6A-D presented the results of SEM imaging on betung bamboo after *P. chrysosporium* pretreatment with the incubation time of 30 and 45 days. This figure showed that the fungi colonized the chips, and degraded cell walls of bamboo and detached the fibers. Partial degradation of cell lumen walls was evident. There was no substantial difference of the degradation levels within these treatments. Therefore, to clearly identify the changes on the cell walls degradation, further examination using the ultra-structural technique is necessary. This cell walls degradation using different white rot fungi (i.e. *C. subvermispora*) has been also reported in previous studies (Akhtar *et al*. 1998).

It showed that there was a substantial effect on wood cell walls or modification of cell wall within a relatively short time after inoculation, although amount of lignin was not significantly removed. Other study on 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 (Akhtar *et al*. 1998).

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Scoring value

Scoring value of betung bamboo pulp characteristic pretreated by *P. chrysosporium* both in Kraft and soda pulping are presented in Table 1. This table indicates a different highest value, i.e 4. The result showed that the best combination of treatment was 10% CSL in inoculums with 30 days of incubation using Kraft process.

Conclusion

Increasing the amount of CSL added to the inoculums at biochemical process using *P. chrysosporium* generally improved the quality of pulp produced. Treatment combinations resulted in various effect on each parameter observed. The more CSL added with Kraft process gave better results on pulp yield. An increase in yield was obtained by the Kraft cooking process while the Soda process resulted in lower yield. Kappa number of Kraft process showed lower results than those of Soda process especially in the utilization of 1% CSL in inoculums.

Generally, the increasing time of incubation period tend to increase pulp yields and decrease the Kappa numbers. However, pulp freeness and brightness increased especially for Kraft process. Combination of treatments gave low delignification selectivity except for the 1% CSL amount. A further study should be conducted to examine the sharply decreasing of Kappa number and increasing of delignification selectivity in treatment of 1% CSL using Kraft process. The SEM images showed that there were cell walls degradation activities but no substantial difference on degradation levels among different treatments. The best properties of pulp were obtained by the Kraft process for the treatment of 10% CSL with 30 days of incubation time.

Figure 6 SEM image of bamboo pretreated by *P.chrysosporium* (PC 30 days (A) with CSL 1%, PC 45 days (B) with CSL 1%, PC 30 days (C) with CSL 10%, PC 45 days (D) with CSL 10%).

Table 1 Kraft and Soda pulp properties of betung bamboo with pretreatment of *P. chrysosporium* fungi

CSL	Pulping process	Incubation time (days)	Kappa number	Pulp yield (%)	Freeness (ml)	Delignification selectivity (%)	Pulp brightness (% ISO)	Score	Rank
1%	Soda	30	82.31[1]±5.01	28.93[1]±5.63	737.5[5]±6.61	10.21[2]±1.30	7.21[1]±0.10	10	7
		45	77.57[2]±11.13	29.11[2]±3.88	737.5[5]±1.44	10.97[4]±1.74	7.64[2]±0.14	15	6
	Kraft	30	9.29[7]±0.69	36.95[5]±12.33	735[7]±7.07	81.82[7]±11.49	32.31[5]±0.10	31	**3**
		45	9.15[8]±1.09	43.44[7]±5.25	738.33[4]±7.07	83.07[8]±10.35	33.03[6]±0.14	33	**2**
10%	Soda	30	73.12[3] ±3.28	34.30[3]±4.76	735.63[6] ±4.42	9.55[1]±0.62	15.40[3]±0.15	17	5
		45	67.29[4] ±3.68	35.68[4]±3.66	740.63[3]±2.65	10.53[3]±1.20	15.00[4]±0.14	17	5
	Kraft	30	51.59[6]±4.67	44.65[8]±0.97	733.1[8]±22.86	14.01[6]±1.43	33.76[7]±0.10	**35**	**1**
		45	56.04[5]±8.21	40.95[6]±2.81	748.1[2]±5.54	12.95[5]±2.05	40.21[8]±0.19	26	4

References

Akhtar M, Attrid Ge, Myers G, Kirk TK. 1992. Biomechanical pulping of loblolly pine chips with different strains of the white-rot fungus *Ceriporiopsis subvermisphora*. *Tappi J.* 75(2):105-109.

Akhtar M, Attridge MC, Myers GC, Blanchette RA. 1993. Biomechanical pulping of labolly pine chips with selected white rot fungi. *Holforschung* 47:36-40.

Akhtar M, Blanchette RA, Kirk TK. 1997a. *Advances in Biochemical Engineering and Biotechnology*. Berlin: Springer-Verlag. Pp. 127.

Akhtar M, Blanchette RA, Myers GC, Kirk TK. 1997b. An overview of Biomechanical Pulping Research. In. Young, RA, Akhtar M, editors. *Environmentally Friendly Technologies for The Pulp and Paper Industry*. New York: John Wiley & Sons, Inc.

Akhtar M, Lentz MJ, Blanchette RA, Kirk TK.1997c. Corn steep liquor lowers the amount of inoculum for biopulping. Peer reviewed, *Tappi J.* 80(6):161-164.

Akhtar M, Scott GM, Swaney RE, Kirk TK. 1998. Overview of Biomechanichal and Biochemichal Pulping Research in: Eriksson K, Cavaco-Paulo A, editor. *Enzyme Applications in Fiber Processing*. Washington DC: American Chemichal Society.

Akhtar M, Scott GM, Swaney RE, Shipley DF. 2000. Biomechanichal pulping: a mill-scale evaluation. *Res Conserv. Recycling 28*:241-252.

Atchison JE. 1998. Update on globa; use of non-wood plant fibers and some prospects for their greater use in the United States. In: *Proceedings of the TAPPI North American Non-Wood Fiber Symposium*. Pp13-42

Ates S, Ni Y, Atik C, Imamoglu I. 2008a. Pretreatment by *Ceriporiopsis subvesmispora* and *Phlebia subserialis* of wheat straw and its impact on subsequent soda AQ and kraft AQ pulping. *Roumanian Biotechnol. Lett.* 13 (5):3914-3921.

Ates S, Atik C, Ni Y, Gumuşkaya E. 2008b. Comparison of different chemical pulps from wheat straw and bleaching with xylanase pre-Treated ECF method. *Turk. J Agri. For.* 32:561-570.

Bar-Lev SS, Kirk,TK, Chang H. 1982. Fungal treatment can reduce energy requirments for secondary refining of TMP. *Tappi J.* 65:111-113.

Behrendt CJ, Blanchette RA, Akhtar M, Enebak SA, Iverson S, Williams DP. 2000. Biomechanichal pulping with *Phlebiopsis gigantea* reduced energy consumption and increased paper strength. *Tappi J.* 83(9):1-8.

Blanchette, RA. 1991. Delignification by wood-decay fungi. *Annu. Rev. Phytopathol.* 29:381-398

Blanchette, RA. 1995. Degradation of the lignocellulose complex in wood. *Can. J. Bot*. 73:S999-S1010.

Copur Y, Tozluoglu A. 2007. The effect of AQ and $NaBH_4$ on bio-kraft delignification (*Ceriporiopsis subvermispora*) of brutia pine chips. *Int.. Biodet. Biodeg.* 60:126–131.

Fatriasari W, Hermiati E. 2008. Analisis morfologi serat dan sifat fisis-kimia pada enam jenis bambu sebagai bahan baku pulp dan kertas. *JITHH* 1(2):67-72.

Fatriasari W, Ermawar RA, Falah F, Yanto DHY, Hermiati E. 2009**.** Pulping soda panas terbuka bambu betung dengan praperlakuan fungi pelapuk putih (*Pleurotus ostreatus* dan *Trametes versicolor)*. *JITHH* 2(2):45-50.

Fatriasari W, Ermawar RA, Falah F, Yanto DHY, Adi DTN, Anita SH, Hermiati E. 2010. Biopulping of betung bamboo using white rot fungi (*P.ostreatus* and *T.versicolor*) with kraft and soda processes. *J Ilmu dan Teknologi Kayu Tropis* 8 (2):121-133.

Fengel D, Wegener G. 1989. *Wood: Chemistry, Ultrastructure, Reaction*. Berlin: Walter de Gruyter.

Ferraz A, Parra C, Freer J, Baeza J, Rodriguez J. 2002. Characterization of white zones produced on Pinus radiata wood chips by Ganoderma australe and Ceriporiopsis subvermisphora. *World J Microbiol. Biotech.* 16:641-645.

Fitria, Ermawar RA, Fatriasari W, Fajriutami T, Yanto DHY, Falah F, Hermiati E. 2006. *Biopulping Bambu Menggunakan Jamur Pelapuk Putih Schizophylum commune.* Bogor: UPT BPP Biomaterial

Hakalla TK, Lundella T, Galkina S, Maijalaa P, Kalkkinenb N, Hatakkaa A. 2005. Manganase peroxidases, laccases and oxalic acid from the selective white-rot fungus *Physisporius rivulosus* grown on spruce wood chips. *Enzyme Microbial. Technol.* 6:462-468.

Hatakka A. 2001. Biodegradation of Lignin. In: *Biopolymer, Biology, Chemistry, Biotechnology, Application*. *Vol 1. Lignin, Humic Substances and Coal,* Hofrichter A, Steinbuchel A, editors. Berlin: Wiley-WCH. Pp 129-180.

Hatakka A, Hammel KE. 2010. Fungal Biodegradation of Lignocelluloses. In: *The Mycota, A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research*, Esser K Hofrichter M, Editors. Berlin: Springer Heidelberg. Pp 319-340.

Hon DNS. 1996. *Chemical Modification Of Lignocellulosic Materials*. New York: Marchel Dekker,Inc.

Humar M, Pohleven F. 2005. Influence of a nitrogen supplement on the growth of wood decay fungi and decay of wood. *Int. Biodet. Biodeg.* 56:34–39.

Hunt C, Kenealy W, Horn E, Houtman C. 2004. A Biopulping mechanism: creation of acid groups on fiber. *Holzforschung* 58:434-439.

Islam MN, Karim MR, Malinen RO. 2008. Beneficial effects of fungal treatment before pulping and bleaching of *Acacia mangium* and *Eucalyptus camaldulensis*. *Turk. J Agric. For.* 32:331-338.

Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe T. 2003. Bioorganosolve pretreatment for simultaneous saccarification and fermentation of beech wood by ethanolysis and white rot fungi. *J Biotech. 103*:273-280.

Karlsson H, Lorentzen AB, Wettre. 2006. *Fibre Guide: Fibre Analysis and Process Applications in The Pulp and Paper Industry*. Kista: AB Lorentzen & Wettre.

Kirk TK, Higuchi T, Chang H. 1980. Lignin Biodegradation: Summary and Perspectives. In: Kirk TK, Higuchi T, Chang H, editors. *Lignin Biodegradation Microbiology, Chemistry, and Potential Application,* vol 2. Boca Roton: CRC Press Inc.

Kirk TK, Tien M, Kersten PJ, Mozuch MD, Kalyanaramant B. 1986. Ligninase of *Phanerochaete chrysosporium*: mechanism of its degradation of the non-phenolic arylglycerol β-aryl ether substructure of lignin. *Biochem. J.* 236:279-287.

Leatham GF, Kirk TK. 1982. Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. *FEMS Microbiol. Lett.* 16:65-67.

Leatham GF, Myers GC, Wegner TH, Blanchette RA. 1990. Biomechanical pulping of aspen chips: paper strength and optical properties resulting from different fungal treatments. *Tappi J.* 73(3):249-255.

Lee AWC, Chen G, Trainter FH. 2001. Comparative treatibility of moso bamboo and southern pine with CCA preservative using a comercial schedule. *Biores. Technol.* 77:87-88.

Messner K, Srebotnik E. 1994. Biopulping, an overview of developments in an environmentally safe paper-making technology. *FEMS Microbiol. Lett.* 13:351-364.

Messner K, Koller K, Wall MB, Akhtar A, Scott GM. 1998. Fungal Treatment of Wood Chips for Chemical Pulping. In: *Environmentally Friendly Technologies for the Pulp and Paper Industry*. New York: John Wiley and Sons Inc. Pp 385-398.

Mosai S, Wolfaardt JF, Prior BA, Christov LP. 1999. Evaluation of selected white-rot fungi for biosulfite pulping. *Biores. Technol.* 68: 89–93.

Oriaran TP, Labosky P, Blankenhorn PR. 1990. Kraft pulp and papermaking properties of Phanerochaete chrysosporium degraded aspen. *Tappi J.* 73:147-152.

Perez J, Dorado JM, Rubia TD, Martinez J. 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *Microbiol.* 5:53-63.

Rajangam A. 2005. Functional genomics of wood degradation and biosynthesis licentiate. [Thesis]. Stockholm: School of Biotechnology. Royal Institute of Technology.

Ramos J, Gonzalez M, Ramirez F, Young R, Zuniga V. 2001. Biomechanical and biochemical pulping of sugarcane bagasse with *Ceriporiopsis subvermispora* fungal and xylanase pretreatments. *J Agric. Food Chem.* 49:1180-1186.

Reid ID. 1998. Bleaching kraft pulps with with white-rot fungi. In: Young R, Akhtar M, Editor. *Environmentally Friendly Technologies for the Pulp and Paper Industry*. New York: John Willey. Pp 505-514.

Setliff EC, Marton R, Granzow SG, Eriksson KL. 1990. Biomechanical pulping with white-rot fungi. *Tappi J.* 73:141-147.

Shukla OP. 2004. Biopulping and biobleaching: an energy and environment saving technology for Indian pulp and paper Industry. *Enviro News Newsletter of ISEB India* 10 (2).

Sjostrom E. 1995. *Kimia Kayu, Dasar-dasar dan Penggunaan*. Edisi 2. Sastrohamidjojo H, penerjemah. Prawirohadmodjo S, editor. Yogyakarta: Gadjah Mada University Press. Terjemahan dari: *Wood Chemistry, Fundamentals and Applications.*

Smook GA. 1992. *Handbook for Pulp & Paper Technologists*. Second Edition. New York: Angus Wilde Publications

Valkomies J. 1969. Wood raw materials for pulp paper in tropical countries. An International Review of Forestry and Forest Products. *Unasylva* 23(3):94.

Vu THM., H. Pakkanen, R. Alen. 2004. Delignification of bamboo (*Bambusa procera acher*) Part 1. Kraft pulping and the subsequent oxygen delignification to pulp with a low kappa number. *J Industrial Crops Prod. Int.* 19:49-50.

Yaghoubi, Pazouki M, Shojaosadati SA. 2008. Variable optimization for biopulping of agricultural residues by *Ceriporiopsis subvermispora*. *Biores. Technol.* 99:4321-4328.

Zhang X, Huang H, Liu Y. 2007. Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms. *Int. Biodet. Biodeg.* 60:159–164.

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