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Original Article

Increased delignification rate of *Dendrocalamus strictus* (Roxburgh) nees by *Schizophyllum commune* Fr.; Fr. to reduce chemical consumption during pulping process

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Abstract

Pulp and paper industry is traditionally known to be a large contributor to environmental pollution due its large consumption of energy and chemicals. To reduce the chemical consumption, rate of delignification was increased by *Schizophyllum commune* in destructured sample of *Dendrocalamus stictus,* which was destructured by Impressafiner (compression-cum dewatering process). The extent of delignification was determined and comparison was made between the non-destructured and destructured samples. The influence of physical parameters like incubation time, moisture level, media, media concentration, pH and temperature were also examined during the study. It was found that rate of delignification was significantly 6.43% more in destructured sample than non-destructured sample. Kraft pulping of treated destructured sample shows 2.59 point reduction in kappa number than untreated non-destructured sample. Thus this paper provides an insight of the delignification extent in *Dendrocalamus strictus* after mechanical operation at varying physical parameters.

Keywords: biodelignification, kraft pulping, lignin, white rot fungi, holocellulose

1. Introduction

The pulp and paper industry is one of the oldest and core industrial sectors of India. The socio-economic importance of paper has its own value to the country's development as it is directly related to the industrial and economic growth of the country. Fibers for paper are isolated from the wood and/or agro-based raw materials using conventional mechanical or chemical methods (Sridach, 2010). Formation of paper from these fibers, considered as an art up to the end of the nineteenth century, has now been fully mechanized. The pulp and paper sector is among one of the most energy intensive and highly polluting industrial sectors of the Indian economy and is therefore of particular interest in the context of both local and global environmental concerns. Demand for paper is expected to rise in the future with increasing literacy and growth of the manufacturing sector in India. A large number of capacity expansion and modernization initiatives in the sector are already planned by the mills to meet the growing demand; however, intervention of biotechnological processes to increase productivity by adoption of innovative, efficient and cleaner process is desirable to address the economic, environmental and social development issues.

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Raw materials, energy, chemicals, labor and water are the major inputs for the production of paper. Raw materials and energy constitute about 50-60% of the total cost of production and these are the major variables affecting the cost of production. The prices of raw materials and energy have escalated during last decades and are bound to increase further. The consumption of chemical cost component varies from 10-12% and is also significant in terms of its impact on pollution and subsequent cost of pollution abatement.

Optimal use of raw materials, energy and chemicals is necessary in order to compete in open economy and therefore major research worldwide is focused on their efficient utilization and resource conservation. Optimum chemical and energy usage without compromising the quality of the products is a major challenge among researchers; therefore, efforts are being made to address these issues. The paper highlights possibility to reduce chemical consumption by adoption of biotechnological approaches for more delignification.

Biotechnology has capabilities to provide new solutions to pulp and paper industry to alleviate environmental impact and reduce investment cost. Biological pretreatment is also known as biopulping in pulp and paper technology, and has potential to overcome some of the problems associated with mechanically manufactured pulp (Leatham *et al.*, 1990; Kirk *et al.*, 1993; Berrocal *et al.*, 2004) and decrease chemical consumption in chemical pulping operations (Messner and Srebotnik, 1994). Biopulping is an eco-friendly technology and also has potential to reduce electrical energy consumption as well as avoid pollution by reducing the chemicals used in chemical pulping (Okano *et al.*, 2005). Recent reports show that a biologically based approach has potential for improving the quality of paper, economics and environmental impact of pulp generation (Scott *et al.*, 1998; Behrendt *et al.*, 2000; Kenealy *et al.*, 2004).

The main biological challenge in biopuling is that fungal hyphae and their lignolytic enzymes (large molecular size) are not able to penetrate the core of chips only surface phenomenon occurs during treatment stage and lignin is solubilized only superficially (Blanchette *et al.*, 1997). Considering this phenomenon, bamboo chips were mechanically destructured for opening the compact fibers thereby converting them to a spongy material to increase the accessibility of the interior of the fiber to the fungal hyphae and its lignolytic enzymes. Our objective in the present paper was to increase the rate of delignification by using novel sample modification techniques.

2. Materials and Methods

2.1 Fungal culture

The freeze-dried white rot fungus *Schizophyllum commune* was obtained from National Type Culture Collection (NTCC-1139), Forest Pathology Division of the Forest Research Institute, Dehradun. The culture was maintained on potato dextrose agar media (PDA) slants and kept refrigerated until used. Cultures from the slants were inoculated on PDA plates and incubated at 27±1°C for 7 days.

2.2 Suspension culture preparation

Active inocula from these plates were grown in 250 ml Erlenmeyer flasks containing 100 ml malt extract broth. The inoculated flasks were incubated without agitation in an incubator at 25±1°C for 7 days. The surface of the medium got covered with the fungus in the form of mat. The fungal mat was removed from the medium and suspended in sterilized distilled water. The fungal mat was converted into uniform suspension by using magnetic stirrer at high speed. This suspension was used to inoculate the bamboo samples.

2.3 Sample preparation

Two different forms of bamboo samples were taken for the experiments i.e. non-destructured and destructured. Non-destructured sample was prepared with the help of laboratory chipper and destructured sample was prepared with the help of impressafiner. This is a locally fabricated device (designed by Forest Research Institute, Dehradun) which is used for separating fibers from chips. Figure 1 shows Schematic Layout of Compression Cum Dewatering Unit (impressafiner) drawn by the institute to explain their working mechanism. This unit completely compresses the chips and squeezes out soluble material along with water. Before passing through impressafiner, soaking of bamboo chips was carried out in water for overnight. The soaked chips after draining were dewatered in a compression-cum-dewatering unit (Impressafiner) at 8 r.p.m and 6000 psi. The spongy destructured and non-destructured bamboo samples were dried in sunlight to the normal moisture content and packed in polyethylene bags for further experiment.

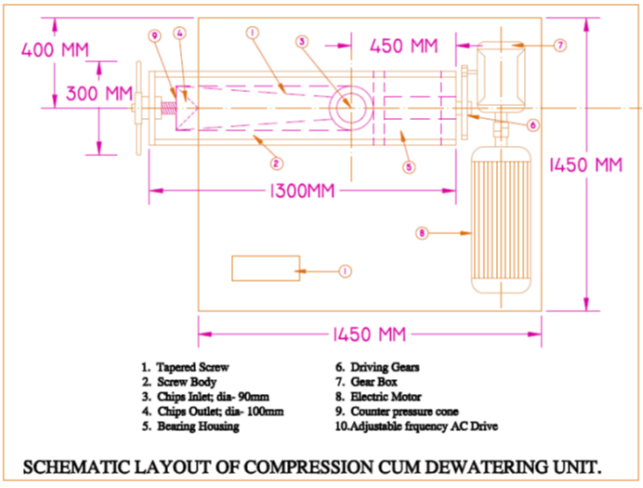


Figure 1. Schematic layout of compression cum dewatering unit (Impressafiner).

2.4 Inoculation procedure

The biodelignification of bamboo samples was performed in 1000 ml conical flasks, containing 50 g (O.D. basis) destructured and non-destructured samples separately. Distilled water was added to the samples in sufficient quantity to increase the moisture level up to 60% - 100% on the dry weight basis for optimum growth of the fungus. Nutrients malt extract broth and molasses solution having different initial pH (4.5–7.0) values were added at different concentration (2-10%) to the raw material and mixed well. Conical flasks were autoclaved for 20 minutes at 121°C. The autoclaved destructured and non-destructured samples were inoculated with mycelium suspension of *Schizophyllum commune*. The rate of mycelium application was 0.003 g (O.D. basis). Each conical flask with sufficient aeration was placed in an incubator at different temperatures (20°C-35°C) for successive time periods. Experiments were performed to obtain the best conditions for delignification by using the selected fungal culture. Effect of each variable on delignification was studied after keeping the other variables constant. Further experiments were conducted on the optimum conditions obtained after a trial of experiments.

The biologically delignified samples obtained after specified biodelignification time were thoroughly washed with distilled water. The washed samples thus obtained were dried in oven at 50-60°C for 48 hrs. Dust was prepared from dried sample of *Dendrocalamus strictus* by using willy mill and the dust passing through 40 mesh and retained over 60 mesh was used for analysis of lignin and holocellulose. TAPPI standard methods were adopted for proximate chemical analysis.

2.5 Cooking process and conditions

The treated and untreated bamboo destructured and non-destructured samples were cooked by kraft pulping process in a laboratory digester consisting of six autoclaves rotating in an electrically heated poly-ethylene glycol (PEG) bath. Before cooking the moisture contents of the bamboo samples were carefully determined using a representative sample. The known weight of samples (200 g O.D.) was charged in each autoclave with an appropriate amount of white liquor of 25% sulfidity and 16% active alkalinity at 1:4 ratios of raw material and liquid. The schedule of digester heating consisted of 30 min for heating from ambient temperature to 100°C, 90 min for heating from 100°C to 160°C. The cooking time at 160°C was 90 minutes. Washing was carried out with warm water and followed by mechanical disintegrator to disintegrate the pulp samples. After washing the pulp yield was determined. After calculating yield, pulp was screened on flat 0.20 mm slotted screen, in order to separate the undesired materials (reject %) from the pulp. The kappa number of pulp was measured by using TAPPI standard method T236 cm-76. Hand sheets (60 gsm) were prepared of both treated and untreated bamboo pulps with the help of laboratory sheet former to determine physical strength properties.

2.6 Statistical analysis

Experiments were performed in triplicate, and results are presented as mean ± S.E. (standard error). Possible treatment differences among the incubation time, moisture, media, media concentration, pH and temperature were explored by the analysis of variance (ANOVA) and Duncan multiple range test (DMRT) by running SPSS and Excel software. DMRT was used to find significant differences among treatments, if any.

3. Results and Discussion

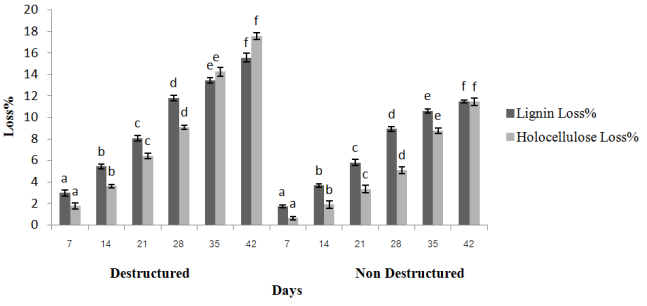
3.1 Chemical analysis

The chemical analysis of both bamboo (non-destructured and destructured) samples was done by Tappi Useful Method 249 and T 222 om-88 for holocellulose and Klason lignin respectively. The lignin content of non-destructured and destructured was found to be 28.15% and 27.50% respectively. The total holocellulose content was 67.85% and 69.53% respectively.

3.2 Effects of varying growth parameters on rate of biodelignification by the *Schizophyllum commune*

3.2.1 Effect of time on biodelignification

Figure 2 depicts the effect of incubation time period on lignin and holocellulose degradation by *Schizophyllum commune* in destructured and non-destructured samples. The fungal inoculated conical flasks were incubated for a period of 42 days and the decrease in lignin and holocellulose content of the substrate was analyzed after a regular interval of 7 days (i.e., 7, 14, 21, 28, 35 and 42 days). Analysis



Means with similar superscript are not significantly different (P> 0.05) from each other. Duncan's multiple range test with level of significance = 0.05.

Figure 2. Effect of incubation period (days) on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune*.

of variance of data showed significant difference between lignin and holocellulose degradation in destructured and non-destructured samples by varying incubation periods. Results of Duncan's multiple range test revealed significant difference ($p<0.05$) between all the treatment means.

It was observed that with increase in incubation time the lignin content decreased in both destructured and non-destructured samples due to growth of fungus. The maximum lignin loss was observed between the incubation period of 21 to 28 days when it went up from 8.06% to 11.82% i.e. an increase of 3.76% in destructured samples, whereas the value was 3.14% when it went up from 5.80% to 8.94% in case of non-destructured samples at the same incubation period. After 28 days of incubation, it was observed that rate of delignification decreased.

The holocellulose loss (%) increased rapidly from 9.08% to 14.26% showing a sudden increase in loss up to 5.18% in destructured samples and 5.08% to 8.79% showing a sudden increase of 3.71% in non-destructured samples, which was much greater in comparison to lignin loss. On the basis of above results, 28 days has considered as the optimum incubation period for fermentation without a significant loss of holocellulose.

3.2.2 Effect of moisture on biodelignification

Effect of different moisture level on biodelignification was observed by adding distilled water at the rate of 60%, 80% and 100% for both non-destructured and destructured samples. Figure 3 shows an increase in lignin loss up to 100% moisture level in case of destructured samples. However, in the case of non-destructured samples maximum loss was noted at 60% moisture level. The maximum lignin loss (%) in the case of destructured samples was 14.06%, whereas in the case of non-destructured samples the lignin loss (%) decreased with increasing moisture level. The decrease in lignin loss with increasing moisture levels can be explained by the fact that extra water present in non-destructured samples inhibited the fungal growth.

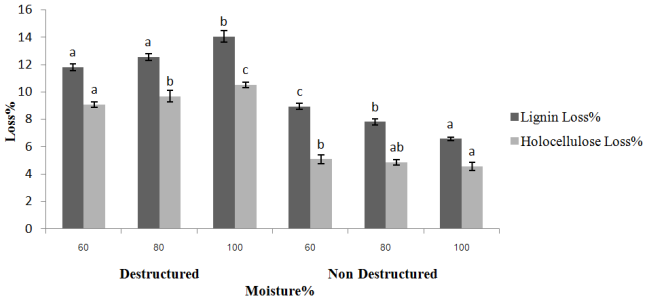


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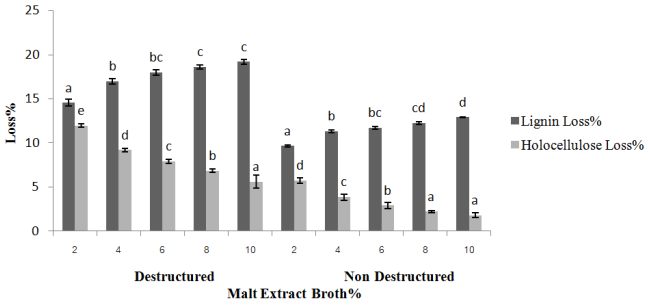
Figure 3. Effect of moisture (%) on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune.*

On the basis of above observation, 60% and 100% moisture level were taken as optimum for non-destructured and destructured samples respectively to conduct further experiments. At optimum moisture level holocellulose were 10.52% and 5.08% in destructured and non-destructured samples. Analysis of variance of data showed significant difference between lignin and holocellulose degradation in destructured and non-destructured samples with varying moisture level. The difference in the optimum conditions might be due to the open structure of the wood which results in more water absorbance in destructured samples than non destructured samples.

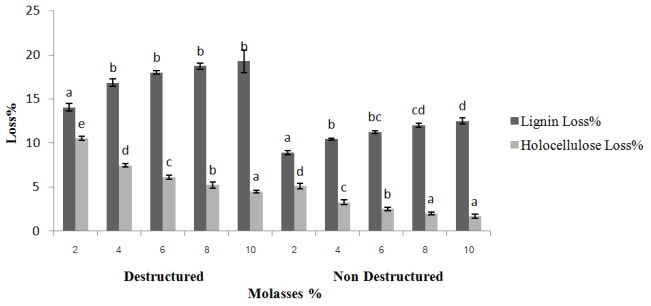
3.2.3 Effect of media and media concentration on biodelignification

Experiments were performed using malt extract broth and molasses separately with different concentration i.e. 2%, 4%, 6%, 8% and 10%. Figures 4 and 5 depict effect of media concentrations of both malt extract broth (MEB) and molasses on lignin and holocellulose degradation by *Schizophyllum commune* in destructured and non-destructured



Means with similar superscript are not significantly different (P> 0.05) from each other. Duncan's multiple range test with level of significance = 0.05.

Figure 4. Effect of malt extract broth (%) on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune.*



Means with similar superscript are not significantly different (P> 0.05) from each other. Duncan's multiple range test with level of significance = 0.05.

Figure 5. Effect of molasses (%) on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune.*

samples. The results from both destructured and non-destructured samples indicated that with increased medium concentration, lignin degradation increases and holocellulose degradation decreases. A sharp increase in lignin loss was observed when concentration of both the media was increased from 2% to 4%. The observed loss was 2.42% (14.55 to 16.97%) in destructured and 1.66% (9.65 to 11.31%) in non destructured samples using malt extract broth. However, it was 2.79% (14.06 to 16.85%) in destructured and 1.48% (8.94 to 10.42%) in non-destructured samples with molasses. On increasing the concentration from 4% to 10%, lignin content showed very little change. Analysis of variance of data showed significant difference between lignin and holocellulose degradation in destructured and non-destructured samples with varying medium concentration of both malt extract broth and molasses.

On supplementation of 4% media in the case of malt extract broth; holocellulose loss was 9.18% in destructured and 3.86% in non-destructured samples. However, in the case of molasses, loss in holocellulose was 7.45% in destructured and 3.27% in non-destructured samples, which was lower in comparison to the loss observed at 2% media concentration. Therefore, 4% was taken as the optimum medium concentration for both the media considering maximum lignin losses with minimum loss in holocellulose. On the basis of above observation, more delignification was found with malt extract broth but if we consider the commercial aspects the molasses are economically 10 times cheaper as compared to malt extract broth. In this view molasses were used to conduct further experiments.

3.2.4 Effect of pH on biodelignification

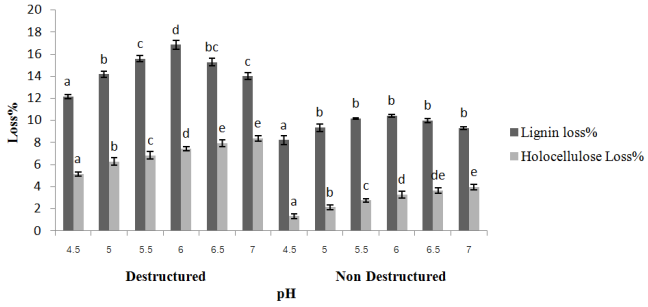
Figure 6 shows the effect of pH on lignin and holocellulose degradation by *Schizophyllum commune* in destructured and non-destructured samples. The maximum lignin loss was observed when initial pH was adjusted to 6.0 for both the samples. The lignin loss was observed to decrease with increase in pH from 6.0 to 7.0 in both the samples. Similar trend was observed by decreasing the pH of the media below 6.0. The holocellulose loss was found to increase with increase in pH from 6.0 to 7.0 in both the samples. However, below pH 6.0 losses in holocellulose started decreasing to pH 4.5. On the basis of above results, pH 6.0 was considered as optimum pH for further experiments. Analysis of variance of data showed significant difference between lignin and holocellulose degradation in destructured and non destructured samples with varying pH.

3.2.5 Effect of temperature on biodelignification

Temperature is an important factor affecting the performance of fungi. Experiments were conducted at four different temperatures i.e. 20°C, 25°C, 30°C and 35°C. The effect of temperature as a variable on lignin and holocellulose loss (%) in fungal treated destructured and non-destructured samples is presented in Figure 7. The maximum lignin loss was observed at 25°C in both destructured and non-destructured samples. The lignin and holocellulose losses were observed to decrease with increase in temperature from 25°C to 35°C in both the samples. However, on decreasing the temperature below 25°C, lignin and holocellulose losses also decreased. Analysis of variance of data showed significant difference between lignin and holocellulose degradation in destructured and non-destructured samples with varying temperature. On the basis of above results 25°C was considered as optimum temperature for both non-destructured and destructured samples.

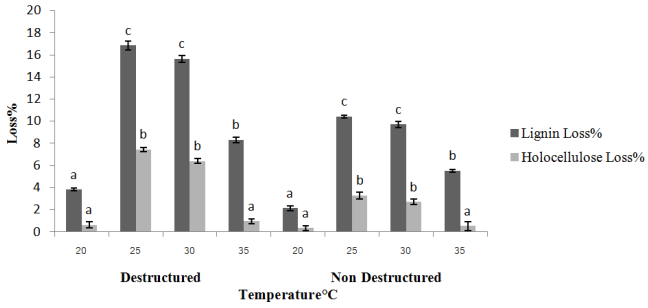
3.3 Pulp characteristics

Table 1 summarizes the results of kraft pulping experiments that were conducted to investigate the effect of fungal treatment on kappa number, pulp yield (%), reject (%), tensile index, tear index and burst index. The investigation has shown that the delignification rate of destructured treated sample (DT) was high, which was represented by decrease in kappa number. The relative rate of delignification for the



Means with similar superscript are not significantly different (P> 0.05) from each other. Duncan's multiple range test with level of significance = 0.05.

Figure 6. Effect of pH on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune.*



Means with similar superscript are not significantly different (P> 0.05) from each other. Duncan's multiple range test with level of significance = 0.05.

Figure 7. Effect of temperature (°C) on lignin and holocellulose degradation in destructured and non-destructured samples by *Schizophyllum commune.*

Table 1. Pulp yield, kappa number and physical strength properties of treated and untreated bamboo samples.

S. no.	Pulp samples	Pulp yield (%)	Kappa number	Rejects (%)	Tensile index (N.m/g)	Tear index ($mN.m^2/g$)	Burst index ($K.Pa.m^2/g$)
1	NDC*	45.32	15.26	0.0820	79.72	20.90	5.45
2	DC **	47.43	14.68	0.0982	72.20	10.88	4.92
3	NDT #	42.59	14.20	0.0447	77.80	16.91	5.92
4	DT##	43.72	12.67	0.0286	68.59	7.49	4.85

*NDC- Non-destructured control,
**DC- Destructured control, #NDT- Non-destructured treated and ##DT- Destructured treated.

samples can be presented as shown below:
Non-destructured sample (NDC) > destructured sample (DC) > non-destructured treated sample (NDT) > destructured treated sample (DT).

After fungal treatment, 2.59 points reduction in kappa number was observed, whereas little difference in pulp yield (%) was observed.

4. Conclusion

Rate of delignification was estimated by applying *Schizophyllum commune* and the comparison was made between the non-destructured and destructured samples. It was found that the extent of delignification was significantly distinct between the two samples. The influence of physical parameters like pH, temperature, media and media concentration, moisture level and incubation time were also optimized during the study. The optimized parameters for destructured sample using *Schizophyllum commune* were 100% moisture, 4% dose of molasses with pH adjusted to 6.0 at 25°C temperature for 28 days of incubation, whereas in the case of non-destructured samples moisture condition was different i.e. 60%. The total lignin loss was 16.85% in destructured samples and 10.42% in non-destructured samples. Kappa number was 2.59 points less in treated destructured sample than in control non-destructured sample. A significant decrease in kappa number would minimize the amount of harsh chemical treatments given for bleaching purpose.

References

Behrendt, C.J., Blanchette, R.A., Akhtar, M., Enebak, S.A., Iverson, S. and Williams, D.P. 2000. Biomechanical pulping with *Phlebiopsis gigantea* reduced energy consumption and increased paper strength. Tappi Journal, 83(9); 1-9.

Berrocal, M.M., Rodriguez, J., Harnandez, M., Perez, M.I., Roncero, M.B., Vidal, T., Ball, A.S. and Arias, M.E. 2004. The analyses of handsheets from wheat straw following solid substrate fermentation by *Streptomyces cyaneus* and soda cooking treatment. Bioresource Technology, 94, 27-31.

Blanchette, R.A, Krueger, E.W., Haight, J.E., Akhtar, M. and Akin, D.E. 1997. Cell wall alteration in loblolly pine wood decayed by the white rot fungus, *Ceriporiopsis subvermispora*. Journal of Biotechnology, 53, 203-213.

Kenealy, W.R., Hunt, C., Horn, E. and Houtman, C. 2004. A new mechanism of biopulping: attachment of acid groups on fiber. In: Ninth International Conference on Biotechnology in the Pulp and Paper Industry, Durban, South Africa, 2004; 10-14 October.

Kirk, T.K., Koning, Jr, J.W., Burgess, R.R., Akhtar, M. and Blanchette, R.A. 1993. Biopulping: a glimpse of the future? U. S. Department of Agriculture Forest Service Research Paper FPL-RP-523. Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, 74 p. Avilable at: www.fpl.fs.fed.us/documnts/fplrp/fplrp523.pdf.

Leatham, G.F., Myers, G.C., Wegner, T.H. and Blanchette, R.A. 1990. Energy savings in biomechanical pulping. In Biotechnology in Pulp and Paper Manufacture, Applications and Fundamental Investigations, T.K. Krik and H.M. Chang, Editors. Butterworths-Heinemann, Boston, pp. 17-25.

Messner, K. and Srebotnik, E. 1994. Biopulping: An overview of developments in an environmentally safe papermaking technology. FEMS Microbiology Reviews, 3 (2-3); 351–364.

Okano, K., Kitagaw, M., Sasaki, Y. and Watanabe, T. 2005. Conversion of Japanese red ceder (*Cryptomeria japonica*) into a feed for ruminants by white-rot basidiomycetes. Animal Feed Science Technology, 120 (3): 235-243.

Scott, G.M., Akhtar, M., Lentz, M.J. and Swaney, R.E. 1998. Engineering, scale-up, and economic aspects of fungal pretreatments of wood chips. In Environmentally Friendly Technologies for the Pulp and Paper Industry, A. Young and M. Akhtar, Editors, John Wiley & Sons, Inc. New York, pp. 341-383.

Sridach, W. 2010. Pulping and paper properties of Palmyra palm fruit fibers. Songklanakarin Journal of Science and Technology, 32 (2); 201-205.