INFLUENCE OF MECHANICAL OPERATION ON THE BIODELIGNIFICATION OF *EUCALYPTUS TERETICORNIS* BY *TRAMETES VERSICOLOR*

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In the biological pulping process, the removal of lignin can be achieved through treatment of lignocellulosic materials with white rot fungi. The main biological challenge in biopulping is that fungal hyphae are not able to penetrate in the core of the chips and only a surface phenomenon occurs. Therefore, the surface area of eucalyptus wood chips was increased by passing through an impressafiner. An impressafiner compresses the chips and converts them into spongy material. In the present study, biological pretreatment of eucalyptus non-destructured (chips) and destructured samples (spongy) was carried out with *Trametes versicolor*. During the study, it was found that the lignin loss was approximately 8.90% higher in the destructured samples, compared to the non-destructured samples, within 21 days under optimum conditions. The fungal pretreatment decreased the kappa number of the treated destructured samples by as much as 10.29 points, compared to the untreated non-destructured samples. Thus, the study has provided an insight into economically feasible conditions to reduce pollution load.

Keywords: biopulping, delignification, white rot fungi, lignin, cellulose

INTRODUCTION

The pulp and paper sector is 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. The demand of paper is expected to rise in the future with an increasing literacy rate and growth of the manufacturing sector in India. The production of paper is based on conventional pulping and bleaching methods. The production of chemical pulps requires a large amount of chemicals that may have a negative environmental impact and mechanical pulping requires a substantial amount of energy.[1,2,3,4] Therefore, research on the development of alternative methods is still being undertaken worldwide. Biodelignification may provide new technologies for the pulp and paper industry to decrease environmental impacts and investment costs.[5,6,7] In this view, wood chips are treated by lignin-degrading fungi, and as a result, the chemical and energy requirement in pulping is decreased and fibre properties of the pulp are improved.[8,9,10,11] However, these processes cannot be applied without modifications. An understanding of the molecular mechanism of biopulping reveals that in biological pretreatments, fungal hyphae are not able to penetrate the core of the chips and only a surface phenomenon occurs during the treatment stage. Due to this, the lignolytic enzymes with large molecular size can solubilise lignin only superficially. Considering this phenomenon, eucalyptus 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 their lignolytic enzymes. Therefore, our objective in the present study was

to increase the rate of delignification in a shorter time by using sample modification techniques. In this study, the biological pretreatment of eucalyptus samples was performed using *Trametes versicolor*.

EXPERIMENTAL

Fungal culture

The freeze-dried white rot fungus *Trametes versicolor* was obtained from Forest Pathology Division, Forest Research Institute, Dehradun. The culture was maintained on potato dextrose agar medium (PDA) slants and kept refrigerated until used. The PDA plate cultures were inoculated from the slants and incubated at 27 ± 1°C for 7 days. The 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 27°C for 7 days. The surface of the medium got covered with the fungus in the form of a mat. The fungal mat was removed from the medium, suspended in sterilized distilled water, and then converted into a uniform suspension by a magnetic stirrer at high speed. This suspension was used to inoculate the wood samples.

Sample preparation

Two different forms of eucalyptus were taken for the experiments, i.e. non-destructured (chips) and destructured. The eucalyptus logs were chopped in a pilot plant chipper. The chips thus obtained were dried in sunlight to the normal moisture content. The destructured samples were obtained by passing the chips through a device called impressafiner (Fig.1). The chips were soaked in water overnight. After draining, the soaked chips were dewatered in a compressioncum dewatering unit (impressafiner) at 8 r.p.m and 6000 psi. This unit completely compresses the chips and squeezes out soluble material along with water. The unit was designed and locally manufactured, handling the capacity of 2-5 kg at one time, to make the material spongy without damaging the fibers. Then the spongy eucalyptus sample was dried in sunlight to the normal moisture content. Both the non-destructured and destructured samples were analyzed for lignin and holocellulose contents using TAPPI standard methods T 222 om-88 and Tappi Useful Method 249 respectively.

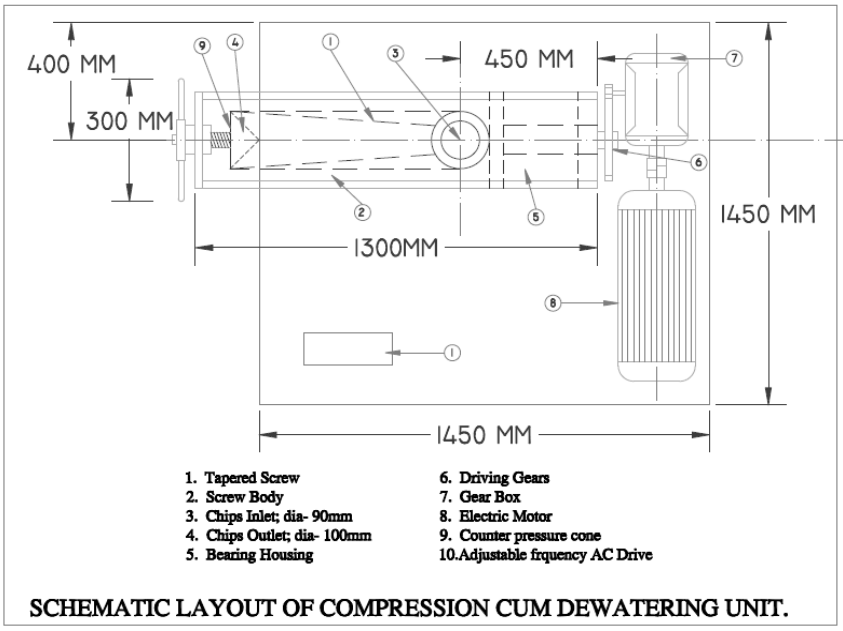

Figure 1: Schematic layout of compression cum dewatering unit (Impressafiner)

Inoculation procedure

The biodelignification of destructured and non-destructured eucalyptus samples was performed in conical flasks, containing 50 g (o.d. basis) samples separately. Distilled water was added to the samples in sufficient quantity to increase the moisture levels from 60 to 100% on a dry weight basis for optimum growth of the fungi. The nutrient malt extract broth and molasses solutions having different initial pH values were added in different doses (2, 4, 6, 8 and 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 (0.003 g on o.d. basis) of *T. versicolor*. The experiments were performed to find the best conditions for delignification by *T. versicolor.* The parameters considered for obtaining the best conditions included incubation time (7, 14, 21 28, 35 and 42 days), moisture (60, 80 and 100%), pH (4.5, 5.0, 5.5, 6.0, 6.5

and 7.0), medium (malt extract broth and molasses), medium dose (2, 4, 6, 8 and 10%) and temperature (20, 25, 30 and 35°C). The effect of each variable on delignification was studied while keeping the others constant.

The initial experiment to study the extent of delignification was carried out for up to 42 days in the incubator. The samples were drawn at a regular interval of 7 days to monitor the rate of delignification. In the initial experiment, the conditions maintained were the following: 60% moisture, 2% medium dose, pH adjusted to 6 and temperature of 25°C. The optimum time conditions found in this experiment were used in the subsequent experiment to find out the effect of moisture by varying it from 60 to 100% at the optimum time period. The nutrient malt extract broth and molasses were added by varying the dose from 2% to 10% to find out the effect of medium and medium dose at the optimum time period and moisture level. In other experiments, the pH and temperature were varied to find out the effect of these variables on delignification. The nutrient solution with initial pH varying from 4.5 to 7.0 was added to find out the effect of pH on delignification at the optimum time period, moisture level, medium and medium dose. The effect of temperature was observed by varying it from 20°C to 35°C at the optimum time period, moisture level, medium, medium dose and pH. For an analytical purpose, conical flasks without inoculum were used as control. Each experiment was done in triplicate. After harvesting, each substrate was oven-dried at 50 to 60 °C for 48 hours. The dried samples of eucalyptus were converted into dust by a Willy Mill and the dust passing through a 40 mesh screen and retained ona 60 mesh screen was used for all subsequent analytical studies. TAPPI T 222 om-88 and Useful Method 249 were used for determination of Klason lignin and holocellulose, respectively.

Cooking process and conditions

The treated and untreated eucalyptus destructured and non-destructured samples were cooked by the 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 content of the wood samples was carefully determined using a representative sample. A known weight of samples (200 g o.d.) was charged into each autoclave with an appropriate amount of white liquor of 25% sulfidity and 16% active alkalinity at a liquid to raw material ratio of 4:1. The schedule of digester heating consisted in 30 minutes for heating from ambient temperature to 100°C and 90 minutes 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 disintegrationof the pulp samples. The washing process continued until the color of the water remained unchanged. After washing, the pulp yield was determined. After calculating the yield, the pulp was screened on a flat 0.20 mm slotted screen, in order to separate the undesired materials (rejects, %) from the pulp. The kappa number of the pulp was measured by using TAPPI standard method T236 cm-76.

Experimental design and statistical analysis

All the experiments were conducted in triplicate and were designed using a Complete Random Design (CRD). The data were subjected to the Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 16. The significant differences among the treatments were compared using Duncan's Multiple Range Tests (DMRT). Treatment values were represented as the mean ± SE.

RESULTS AND DISCUSSION

Chemical analysis

The lignin content of eucalyptus non-destructured and destructured samples was found to be of 34.20% and 33.57%, respectively. The total holocellulose content was 64.38% and 66.25%, respectively.

Effects of varying physical parameters on rates of biodelignification by *Trametes versicolor*

Effect of time on biodelignification

Fig. 2 shows that with the increase in incubation time, the lignin loss increases significantly up to 42 days in both non-destructured and destructured samples, when compared using Duncan's Multiple Range Test (DMRT). Asignificant sharp increase in lignin loss was observed between the incubation period of 14 to 21 days when it went up from 6.53% to 14.28%, i.e. an increase of 7.75%, in non-destructured samples, whereas in destructured samples, when it went up from 11.08% to 19.77%, with an increase of 8.69%. After increasing the incubation period above 21 days, the rate of delignification starts decreasing.The loss in holocellulose after different incubation periods of 7 to 42 days was of 1.64-10.14% in destructured and 0.75-7.97% in non-destructured samples. As observed, the maximum rate of lignin degradation occurred between 14 to 21 days, when the loss of holocellulose was of 3.67% and 5.94% for non-destructured and destructured samples, respectively. The results of DMRT revealed that holocellulose degradation was significantly different for incubation periods from 7 to 42 days in both non-destructured and destructured samples. On the basis of the above observation, 21 days were taken as an optimum

incubation period for both samples to conduct further experiments.

Effect of moisture on biodelignification

The lignin loss (%) observed in the case of the non-destructured samples significantly decreased with increasing the moisture level up to 100%. In the case of the destructured samples, the lignin loss (%) increased significantly up to an 80% moisture level. However, with a further increase in the moisture level, a decrease was observed (Fig. 3). The maximum lignin loss observed was of 14.28% in the non-destructured samples at 60% initial moisture. However, in the destructured samples, the maximum lignin loss was of 22.4% at 80% initial moisture. At the same time, the holocellulose loss recorded was of 3.67% and 6.79% in thenon-destructured and destructured samples, respectively. On the basis of the above observation, 60% and 80% initial moisture levels were taken as optimum for the non-destructured and destructured samples, respectively, to conduct further experiments. The difference in the optimum conditions might be due to the open structure of the wood, which results in a higher water absorbance in the destructured samples than in the non-destructured ones.

Effect of medium and medium dose on biodelignification

As shown in Figs. 4 and 5, lignin loss is increased significantly with the increase in the medium dose from 2% to 4%. A decrease in the lignin loss was observed, in both the non-destructured and destructured samples from 4% to 10%, with both media (molasses or malt extract broth). The maximum lignin loss was observed for 4% medium dose.

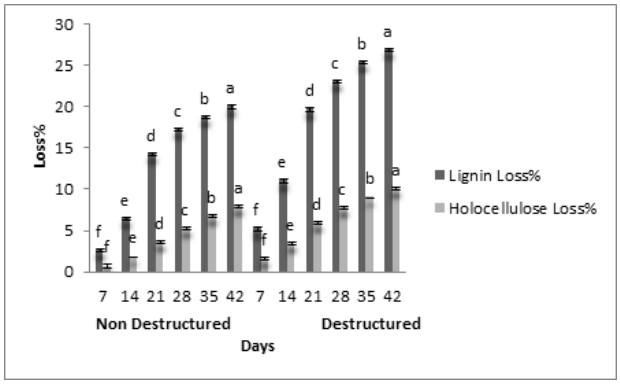

Figure 2: Effect of incubation period (days) on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

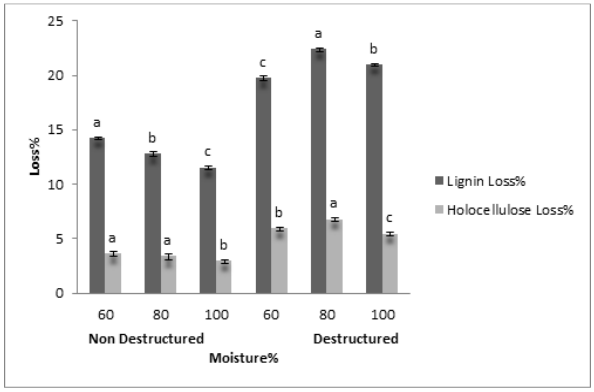

Figure 3: Effect of moisture (%) on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

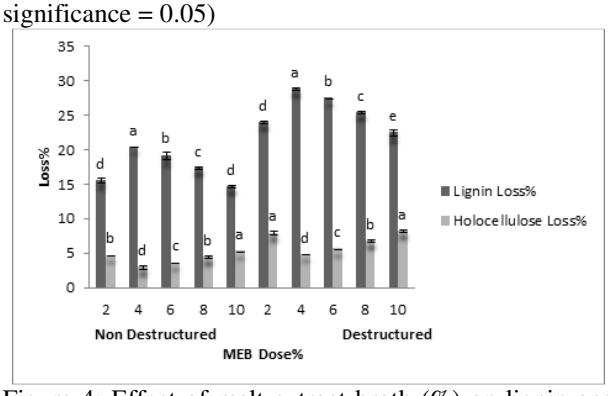

Figure 4: Effect of malt extract broth (%) on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

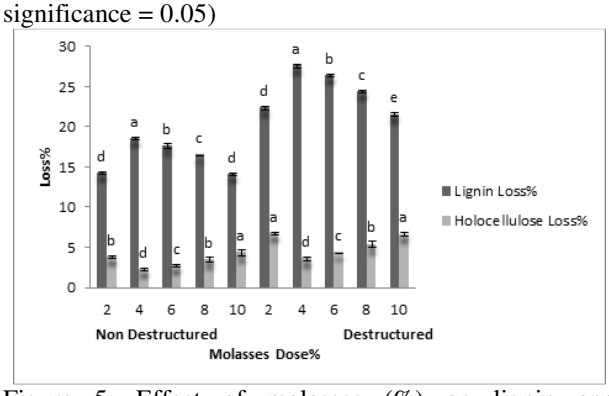

Figure 5: Effect of molasses (%) on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

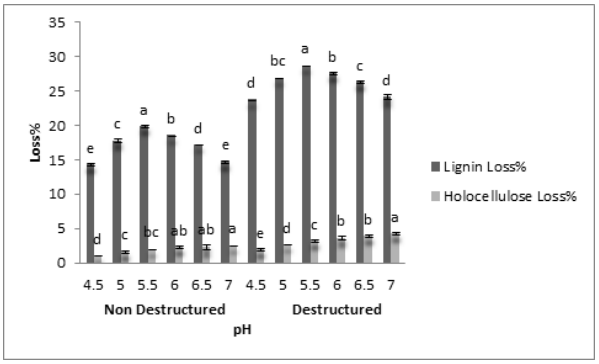

Figure 6: Effect of pH on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

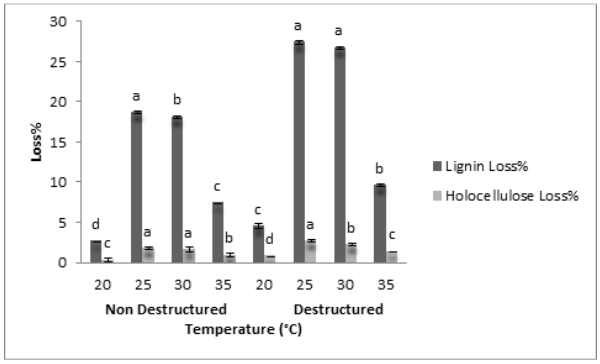

Figure 7: Effect of temperature (°C) on lignin and holocellulose degradation in non-destructured and destructured samples by *Trametes versicolor* (means with similar superscript in columns are non-significant (P>0.05); Duncan's multiple range test with level of significance = 0.05)

The loss in lignin was of 28.88% in destructured and of 20.47% in non-destructured samples, using malt extract broth. However, with molasses, the loss in lignin was of 27.59% in destructured and of 18.62% in non-destructured samples. The results of DMRT reveal that lignin degradation is significantly different for 2 to 10% concentration, for both destructured and non-destructured samples.

A slight difference in the behavior of holocellulose degradation was observed in the presence of any of the two media. The minimum holocellulose degradation was observed at 4% medium dose, but on increasing the dose up to 10%, the holocellulose loss also increased. The minimum loss observed was of 4.88% in destructured and of 2.97% in non-destructured samples, using malt extract broth. Whereas, holocellulose loss observed in destructured samples was of 3.67% and 2.27% in non-destructured samples using molasses.

On the basis of the above observations, more delignification was found with the malt extract broth, but if we consider the commercial aspect, molasses are 10 times cheaper than the malt extract broth. Also, holocellulose loss was comparatively lower in molasses. Considering this, molasses were used to carry out further experiments.

Effect of pH on biodelignification

Fig. 6 shows the effect of pH on lignin and holocellulose degradation by *Trametes versicolor* in destructured and non-destructured samples. The maximum lignin loss was observed when initial pH was adjusted to 5.5 in both types of samples. The lignin loss observed was decreased with the increase in pH from 5.5 to 7.0 in both types of samples. A similar trend was observed by decreasing the pH of the media below 5.5.

The holocellulose loss was increased from pH 6.0 to 7.0 in all the samples. However, below pH 6.0, holocellulose loss was decreased. The results of DMRT reveal that the behavior of destructured and non-destructured samples at pH 5.5 was significantly different. With a view to saving of holocellulose content, pH 5.5 was taken for further experiments.

Effect of temperature on biodelignification

Fig. 7 shows the effect of temperature on lignin and holocellulose degradation by *Trametes versicolor* in both destructured and non-destructured samples. The maximum lignin loss was observed at 25 °C in both types of samples. The lignin and holocellulose losses were observed to decrease with the increase in temperature from 25 °C to 35 °C in all the samples. However, on decreasing the temperature below 25 °C, lignin and holocellulose losses also decreased.

Kraft pulping characteristics

Table 1 summarizes the results of kraft pulping experiments performed to investigate the effect of fungal treatment on kappa number and pulp yield (%). The investigations have shown that the delignification of destructured treated samples (DT) was high, which was represented by the decrease in kappa number. White rot fungi remove and/or modify lignin in wood cell walls, making iteasy to remove during kraft pulping.[12,13] After fungal treatment, a 10.29 point reduction in

kappa number was observed when comparing NDC to DT pulp samples. Whereas, little difference in pulp yield (%) was observed. This decrease may be due to both the dissolution of lignin and the concurrent attack on the carbohydrates.[13,14] A sharp decrease in the rejects % of DT was observed due to swelling and loosening of the cell wall structures after fungal pretreatment.[15,16] The relative rate of delignification for the samples can be presented as shown below: Non-destructured samples (NDC)>destructured samples (DC)>non-destructured treated samples (NDT)>destructured treated samples (DT).

Table 1
Pulp yield and kappa number of treated and untreated eucalyptus non-destructured and destructured samples

S. No.	Pulp samples	Pulp yield (%)	Kappa number	Rejects (%)
1	NDC*	46.15	25.68	5.217
2	DC**	44.69	23.25	2.808
3	NDT#	41.72	21.27	2.298
4	DT##	42.27	15.39	1.644

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

CONCLUSION

The rate of delignification was estimated comparatively by applying *Trametes versicolor* on both non-destructured and destructured eucalyptus samples. It was found that the extent of delignification was significantly different between the two samples. Physical parameters, like pH, temperature, medium, medium dose, moisture level and incubation time, were optimized during the study and their influence was observed. The optimum parameters for the destructured samples using *Trametes versicolor* were found to be 80% moisture, 4% dose of molasses, pH 5.5, 25 °C temperature and 21 days of incubation. Whereas the optimum parameters for the non-destructured samples were 60% moisture, 4% dose of molasses, pH 5.5, 25 °C temperature and 21 days of incubation. The total lignin loss was of 28.78% in the destructured samples and of 19.88% in the non-destructured samples. The kappa number was 10.29 points less in the treated destructured samples than in the non-destructured control samples. This significant decrease in kappa number would minimize the amount of harsh chemical treatments required for the purpose of bleaching.

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