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Pretreatment of Wood Chips and Pulps with *Thelephora Sp.* to reduce Chemical Consumption in Paper Industries

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Abstract: The potential of the white rot fungus, *Thelephora sp***.** and its ligninolytic enzymes in delignification and bleaching of *Eucalyptus grandis* wood chips and Hard Wood Kraft Pulp (HWKP) were evaluated. Hand sheets prepared by conventional chemical methods were maintained as control. The control sheets had 20.0 kappa points and 14.0 ISO brightness. The hand sheets prepared by fungal pretreatment followed by treatment with 50% chemicals (as that of control) had 15.0 kappa points and 16.0 ISO brightness, revealing that *Thelephora sp.* pretreatment could reduce the chemical usage in paper industries without affecting the paper quality.
Key words: *Thelephora sp,* biobleaching, Kappa point, ISO brightness, Ligninolytic enzymes.

Introduction and Experimental

Lignocellulose is the predominant component of woody plant and dead plant materials, and the most abundant biomass on earth[1]. White rot fungi can degrade lignin and a range of environmental pollutants by many of their extra cellular lignolytic enzymes[2]. Removal of lignin from wood is the first step in the manufacturing of chemical paper pulps, kraft alkaline pulping being the most common process[3]. Pulping and bleaching of kraft pulp uses large amounts of chlorine and chloride chemicals. The products of these chemicals are chlorinated organic substances, some of which are toxic, mutagenic, persistent, and bioaccumulating and cause numerous harmful disturbances in biological systems[4]. Pretreatment of wood chips with proper fungi results in significant energy and chemical savings and allows for an improved paper quality[5]. The importance of microbial enzymes in pulp and paper manufacturing has grown significantly in the last two decades[6]. Some lignin-oxidizing enzymes, such as laccases and Mn-peroxidases (MnPs), also showed potential to perform biobleaching reactions by specific lignin oxidation and removal[7]. Biopulping of straw or the treatment of semichemical straw pulp by lignin modifying enzymes would decrease the energy requirement and the consumption of cooking chemicals for lignin removal during pulping[8]. The enzymes responsible for biobleaching are Laccase, Manganese dependent Peroxidase (MnP) and xylanases[9]. The biobleaching system oxidizes the phenolic compound of lignin and the residual lignin is demethylated and significantly enriched in carboxylic acid groups[10]. The white rot fungus *Thelephora sp.* isolated in the present study and its ligninolytic enzymes have been used for pretreatment of *Eucalyptus grandis* wood chips and pulp. The effect of reduced chemical dosage on the quality of the paper obtained from these pretreated pulps was analyzed.

Organism

A portion of *Thelephora sp.* fruit body was surface sterilized with 1% mercury chloride solution, repeatedly washed with sterile distilled water and inoculated on 2% Malt agar plates. The plates were incubated at 37°C for six days for sporulation [11].

Enzyme Preparation

The spore suspension (10^6 spores/ml) prepared from Malt Agar plates were inoculated into C-Limited medium (10%v/v) of the following composition: D-Glucose-3.0g, Diammonium tartarate-0.66g, $MgSo_4.7H_2O$-0.15g, $CaCl_2.2H_2O$-30mg, $FeSO_4.7H_2O$-5.55mg, H_3PO_4(2N)-3.27ml, trace element solution-0.30ml, Vitamin solution-0.30ml and distilled water-1000ml. Trace element solution(g/l): Nitolotriacetate-1.5, $MnSO_4.H_2O$-1.0g, $CoCl_2.6H_2O$-1.0g, $ZnSO_4.7H_2O$-3.0g, $CuSO_4.5H_2O$-3.0g, $Alk(SO_4)_2$-1.0mg, H_3BO_3-10.0mg, Na_2MO_4-10.0mg; vitamin solution (mg/l): biotin-2.0mg, foilc acid-2.0mg, thiamine HCI-50.0mg, riboflavin-5.0mg, pyridoxine HCI-10.0mg, cyanocobalamine-0.1mg, nicotinicacid-5.0mg, calcium pantothenate-5.0mg, p-amino benzoic acid-5.0mg, thioacetic acid 5.0mg. The pH of the medium was adjusted to 4.5 with 4N NaOH or H_2SO_4 solution [12]. The culture flasks were incubated at 30°C for 6 days. After the incubation period, the fungal biomass was removed by filtration and the culture filtrate was centrifuged at 18000rpm for 30 min at 4°C. The clear culture filtrate was partially purified by acetone precipitation (66%v/v) and sephadex G 100 column chromatography. The protein containing fractions were analyzed for Lignin Peroxidase (LiP), Manganese-dependent Peroxidase (MnP) and Laccase activities.

Enzyme assay

Lignin Peroxidase (LiP)

Lignin Peroxidase activity was assayed. The assay solution contained culture filtrate -1.0ml, sodium tartarate buffer (pH 3.5) -100mM, veratryl alcohol-0.4mM, fresh H_2O_2-0.3mM. Immediately after adding H_2O_2, the change in absorbance at 310 nm was recorded at 30 seconds intervals. The enzyme activity was expressed as U/mL (1U= 1µ mole of veratryl alcohol oxidized in 1 min).[13]

Manganese Dependent peroxidase (MnP)

Manganese dependent Peroxidase activity was assayed. The reaction mixture consists of culture filtrate-l.0mL, Phenol red-0.01%, lactate -25mM, $MnSO_4$-100µM, egg albumin-0.1%, H_2O_2-100µM in 1ml of 20mM sodium succinate buffer, pH 4.5. Reactions were carried out at 30°C for 5 min and terminated with addition of NaOH (40µl). The absorbance was measured at 610 nm. The enzyme activity was expressed as U /ml (1U = change in OD/min at 610 nm) [14].

Laccase

Laccase activity was assayed. The reaction mixture consists of culture filtrate-0.5ml, guaiacol-0.35µl (0.035%, v/v), sodium acetate buffer (0.1M; pH 5.0) -2.0ml. The enzyme activity was expressed as change in absorbance at 440 nm and expressed as U/ml (1U= change in OD/min at 440 nm) [15].

Pulping and bleaching of wood chips

Eucalyptus grandis wood chips were used for pulp preparation. The wood chips were cooked in Na_2O solution (1:2.8 w/v) at 170°C for 90 min. Before cooking, the wood chips and the chemicals were preheated at 0-100°C for 30 min and l00-170°C for 80 min. After cooking, the pulp was subjected to alkali-chlorine (EDED) bleaching with a chlorine multiple of 0.18 [16].

Biopulping and bleaching of wood chips

The fungus was grown on wood chips moistened with C-limited medium for 40 days. The chips were then washed thoroughly with water to remove the mycelial growth and cooked as in conventional method with 50% Na_2O concentration, that is, wood chip and Na_2O in the ratio of 1:2.8 and 1:1.4 (w/v) respectively. The cooked pulp was subjected to EDED process with a chlorine multiple of 0.18 and 0.15.

Biobleaching and delignification of hardwood kraft pulp (HWKP)

For biobleaching and delignification of HWKP, whole fungal biomass and the partially purified enzymes (LiP, MnP and laccase) were used. The HWKP was obtained from Tamil Nadu Newsprint and Paper Industry Limited, Karur, India.

Treatment with Fungal Biomass

Mycological broth (200 ml) in a conical flask added with a glass bead (2.5cm diameter) and HWKP (0.25% w/v) was inoculated with fungal spore suspension (10^5 spores/ml) and incubated at 25°C for 5 days on a rotary shaker (200rpm). The resulting suspension was inoculated into HWKP suspended in sterile distilled water (2% w/v) at a concentration of 15% (v/v) and incubated at 25°C for 2 to 5 days on a rotary shaker (200 rpm) [17].

Treatment with enzymes

The HWKP with 2.5% consistency (w/v) was treated with various concentrations (5, 10 and 15 U /ml) of mixed enzyme, LiP, MnP and laccase at 50°C for 24h. For mixed enzyme, LiP and MnP treatments

the pulp suspension was prepared in 20mM sodium succinate buffer, pH 4.5 and for Laccase treatment it was prepared in 0.1 M phosphate buffer, pH 7.0.

Preparation of Hand Sheets

After every pulping and bleaching process, the pulps were thoroughly washed in distilled water and the pulp suspension was filtered through a Bunchner funnel under vacuum. The residue was blotted and air-dried for 24h [18].

Paper Quality

The quality of the paper made was determined in terms of kappa number and ISO brightness points. The pulps were analyzed for kappa number by TAPPI test methods (1993) and brightness of the paper was determined in Perkin Elmerλ3B spectrophotometer equipped with a reflectance sphere at 457nm.

Results and Discussion

Biopulping and Bleaching Of Wood Chips

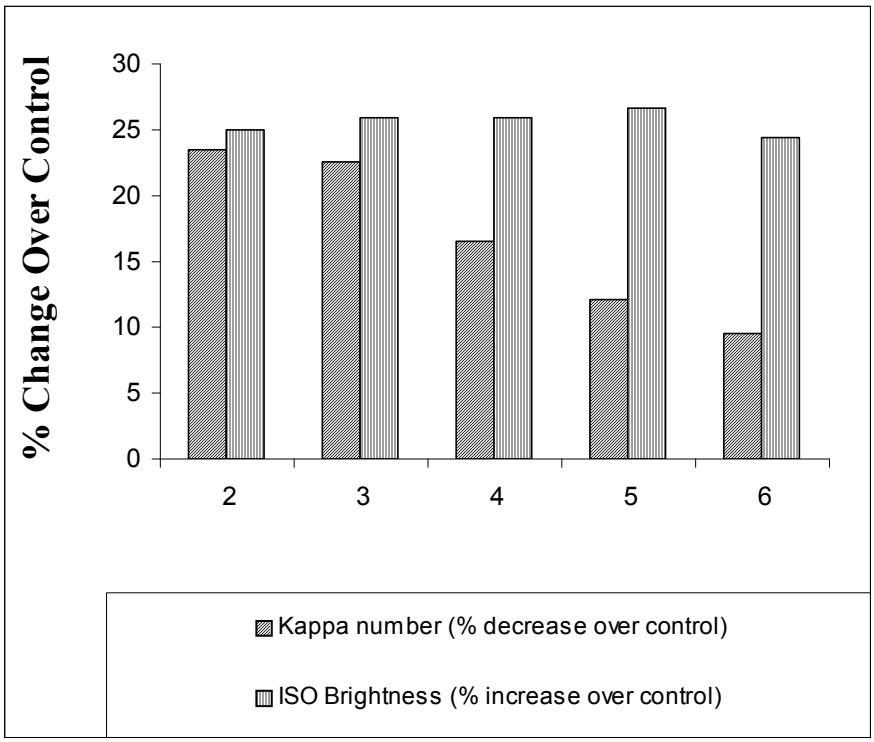
The hand sheets prepared by conventional methods of pulping and bleaching had 20.0 kappa points and 14.0 ISO brightness points. It has been shown that incubation of wood chips with white rot fungi could decrease refining energy requirement and could increase paper quality that incubation of aspen chips for four weeks with *Phlebia brevaspora, Ceriporiopsis subvermispora* and *Dichomictus squalens* decreased the refining energy requirement by 47-68%. [19]Reported pretreated birch wood chips with white rot fungi for four to six weeks prior to pulping; the treatment resulted in a 30 to 50 % reduction in kappa number and 0 to 4% increase in ISO units of brightness. [20] In the present study, observed that pulps obtained from wood chips incubated with *Thelephora sp.* for six weeks followed by conventional cooking had 13.0 kappa points and 16.0 ISO brightness point; when it was further bleached with full dosage of chemicals (chlorine multiple of 0.18), the kappa point was decreased to 9.0 and the ISO brightness point was increased to 19.0. The pulps subjected to biopulping followed by 50% Na_2O treatment had kappa points of 17.0 and ISO brightness point of 16.0. In this step itself, the quality of the biopulp with increased brightness was in par with that of conventionally obtained finished sheets, revealing that pretreatment of wood chips with *Thelephora. Sp.* could reduce the chemical consumption at least by 50% (Table 1).

Table1: Effect of *Thelephora sp*. pretreatment and chemical dosage on paper Quality

Treatment	Kappa number (Points)	Brightness (%) (ISO points)
Conventional chemical method (Control) W: Na2 O (1:2.8 w/v)+0.18 Chlorine multiple	20	14
Biopulping		
FPW: Na_2 O (1:2.8 w/v)	13	16
FPW: Na_2 O (1:1.4 w/v)	17	16
Biobleaching		
FPW: Na_2 O (1:2.8 w/v) + 0.18 chlorine multiple	9	19
FPW: Na_2 O (1:2.8 w/v) + 0.15 chlorine multiple	12	19
FPW: Na_2 O (1:1.4 w/v) + 0.18 chlorine multiple	13	18
FPW: Na_2 O (1:1.4 w/v) + 0.15 chlorine multiple	15	16

W: Wood chip, FPW: Fungal pretreated woodchip

Figure 1. Biobleaching and delignification of Hard Wood Kraft Pulp (HWKP) by Thelephora sp.



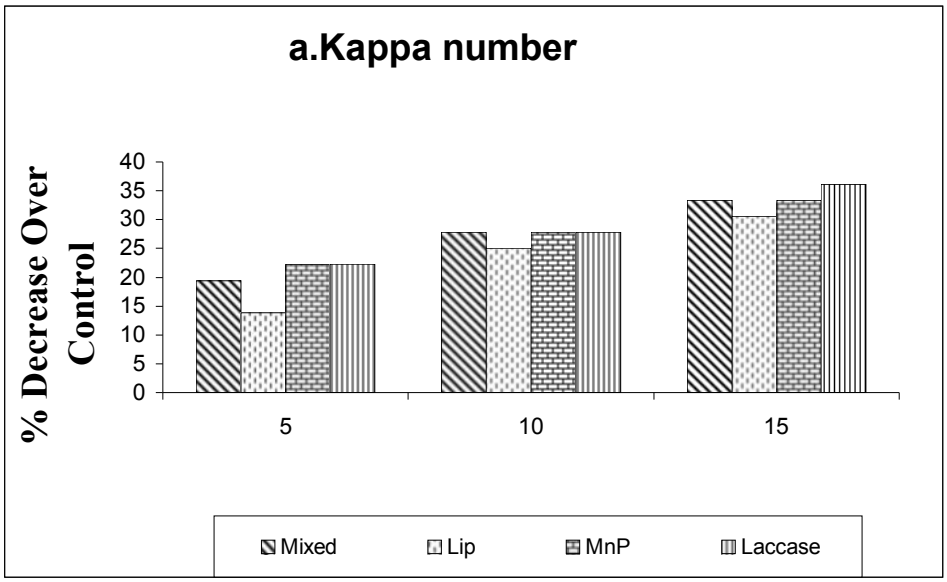
Biobleaching and Delignification of HWKP Treatment with Fungal Biomass

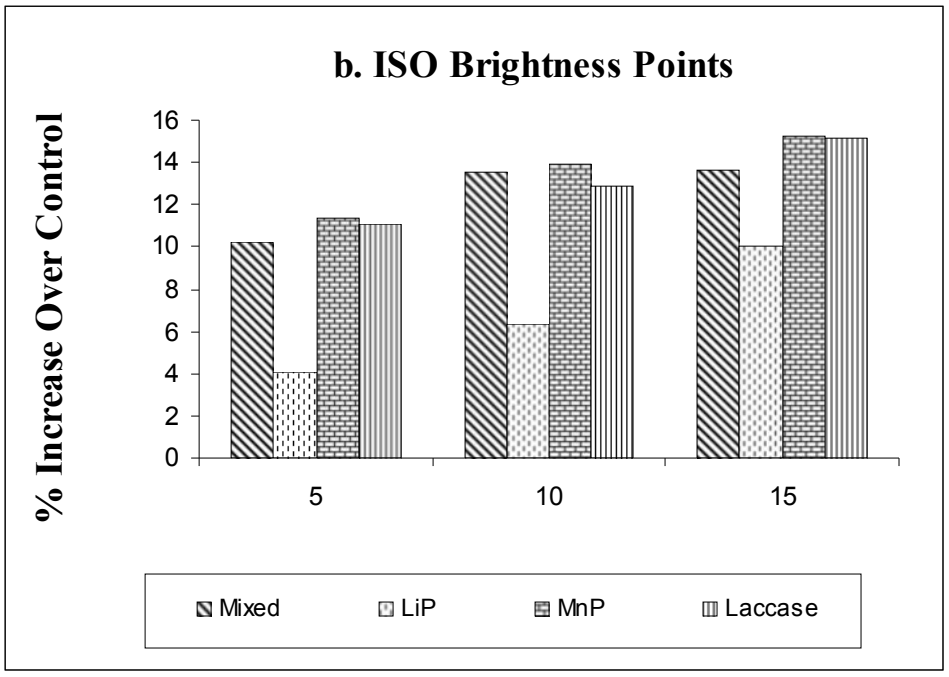
The white rot fungus, *Trametes versicolor* was capable of decolourizing and delignifying the unbleached industrial kraft pulps over 2 to 5 days incubation. The brightness was increased by 28.0 to 45.8% and the kappa number was reduced by 9.56% [21]. *Ceriporiopsis subvermispora* bleached the pulp effectively after 14 days of incubation, the kappa number was decreased from 6.7 to 0.8 and the brightness was increased by 47% [22]. *Bjerkandara sp.* strain BOS55 extensively delignified and bleached the oxygen delignified eucalyptus kraft pulp with a brightness gain of 14.0 ISO units. In the present study it was observed that *Thelephora sp.* could effectively bleach and delignify the HWKP with 63% decrease in kappa number and 29% gain in brightness points after six days (Fig. 1).

Treatment with Enzymes

The Hard Wood Kraft Pulp was treated with Lignin Peroxidase, Manganese Dependent Peroxidase, Laccase and a mixture of these enzymes. Results shown in figure 2 a, b revealed the efficiency of *Thelephora sp.* enzymes in bleaching and delignification of HWKP. At higher concentration (15 U/2.5g dry pulp), the mixed enzymes reduced the kappa number by 33% and increased the brightness by 14%. Lignin Peroxidase decreased the kappa number by 31% and increased the brightness by 10%. In Manganese Dependent Peroxidase treatment, the kappa number was reduced by 33% and brightness was increased by 15%. In Laccase treatment, the kappa number was reduced by 36% and brightness was increased by 15%. Exploitated that ligninolytic enzymes could be employed in the pulp industry for bleaching and delignitication purposes. Especially the enzymes Laccase, Manganese Dependent Peroxidase (MnP) have major role in bleaching process [23]. [24] Reported that treatment of different kinds of pulps with LIGNOZYMS laccase - mediator system (LMS) resulted in 70% reduction in kappa number. [10] Reported that the laccase /N- hydroxybenzotriazole system yielded 52% delignification of soft wood kraft pulp, whereas, use of ABTS yielded 35% delignification. In the present study also, it was observed that Laccase, MnP and the mixed enzyme were very efficient in bleaching and delignifying HWKP. They have decreased the kappa number by 33-36% and increased the brightness by 14-15%. This study is the first to report that *Thelephora sp.* can be used for pretreatment of wood chips and pulps effectively in the paper manufacturing process, to reduce the chemical consumption in paper manufacturing process and to reduce the chemical consumption in paper industries.

Figure 2. Biobleaching and delignification of hard Kraft pulp (HWKP) by Ligninolytic enzymes of Thelephora sp.





Conclusion

The white rot fungus, *Thelephora sp.* and its ligninolytic enzymes in delignification and bleaching of *Eucalyptus grandis* wood chips and Hard Wood Kraft Pulp (HWKP) were evaluated. The hand sheets prepared by fungal pretreatment followed by treatment

with 50% chemicals (as that of control) had 15.0 kappa points and 16.0 ISO brightness, revealing that *Thelephora sp.* pretreatment could reduce the chemical usage in paper industries without affecting the quality of paper.

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