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Research Article

BIOBLEACHING AND DELIGNIFICATION OF HARD WOOD KRAFT PULP (HWKP) BY *TRAMETES* SP., *GANODERMA* SP. AND *PORIA* SP.

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ABSTRACT: In the present study, White rot fungi *Trametes* sp., *Ganoderma* sp. and *Poria* sp. were collected from decayed wood of *Tamirandus indica*, *Eucalyptus grandis* and *Tectonia grandis* respectively from the western ghats region of Tamilnadu and Karnataka in India. The collected fungi were isolated and identified based on the morphological characters from the key provided previously. The collected fungi were examined for biobleaching and delignification of hard wood kraft pulp (HWKP). In biobleaching and delignification of HWKP, all the three fungi reduced the kappa number and increased the brightness of the pulp after 10 days of incubation. The maximum reduction in kappa number (12.76%) and brightness (44.87%) were determined in *Poria* sp.
Keywords: Biobleaching, *Trametes* sp., *Ganoderma* sp. and *Poria* sp . Hard wood kraft pulp (HWKP).

INTRODUCTION

Removal of lignin from wood is the first step in the manufacturing of chemical paper pulps and the most common process [1]. Residual lignin in kraft pulp is highly modified by alkaline condensation reactions during pulping and gives the pulp a characteristic dark brown colour. This residual lignin is commercially removed by bleaching with chlorine based chemicals. The chlorinated products derived from lignin during these bleaching procedures are mutagenic they also cause a waste treatment problem because of their toxicity and dark colour. Therefore, environmental concerns have led us to seek alternative ways to eliminate or at least reduce the use of chlorine based chemicals in bleaching. Increasing awareness about environmental concerns has led the paper industry to look for cleaner production which aimed at the reduced consumption of chlorine and its compounds in the bleaching sequences which thereby minimizes the discharge of chlorinated organic such adsorbable organic halides (AOX) in the effluent [2]. The kappa number is the volume of 0.1N potassium permanganate solution consumed by one gram of moisture free pulp and the results are corrected to 50 per cent consumption of the permanganate added. In recent year's paper mills are adopting eco friendly technologies such as oxygen delignification, enzymatic pre bleaching. Bioleaching has number of advantages such as reduction of AOX levels in discarded effluents and improved pulp quality gain in brightness [3]. Pretreatment of wood chips with proper fungi results in significant energy and chemical savings and allows for an improved paper quality [4]. The importance of microbial enzymes in pulp and paper manufacturing has grown significantly in the last two decades [5]. White rot fungi can degrade lignin and a range of environmental pollutants by many of their extra cellular ligninolytic enzymes. The use of white rot fungi for the biological delignification of wood was first studied at the West Virginia pulp and paper company in the 1950's [6, 7]. The first to recognize that *P. chrysosporium* could partially delignify soft wood unbleached kraft pulp. It was also reported that hardwood unbleached kraft pulp treated with *T. versicolor* showed an increase in brightness and a corresponding decrease in residual lignin concentration [8, 9]. The beneficial effects of bleaching by the white rot fungi *Ceriporiopsis subvermispora, P. chrysosporium* and *Trametes (Coriolus) versicolor.* Several modified kraft pulping methods have been developed over the past 30 years, representing a large improvement in kraft pulping technology. Removal of lignin from wood is the first step in the manufacturing of chemical paper pulps kraft alkaline pulping being the most common process [10]. Pulping and bleaching of kraft pulp uses large amount of chlorine and chloride chemicals the products of these chemical are chlorinates organic substance. Some of which are toxic and mutagenic in the biological systems. Some lignin oxidizing enzymes such as manganese peroxidase and laccase shower the potential for biobleaching reaction by specific lignin oxidation removal by white rot fungi have the significant role in bleach kraft pulp [11].

The bleaching agents used in pulp and paper industries are chlorine, alkali, hypochloride and hydrogen peroxide. The use of chlorine based chemicals in the bleaching process generates chlorophenol compounds which are completely resistant to microbial attack and remain as recalcitrants [12].

MATERIALS AND METHODS

Collection of fungi

The fungi *Trametes* sp., *Ganoderma* sp. and *Poria* sp. were collected from Western Ghats area of Tamilnadu and Karnataka, India and were isolated from decayed wood of *Tamarindus indica, Eucalyptus grandis and Tectonia grandis* respectively. The collection sight was situated in the latitude of -11.58°S and longitude of 76.93°E at 400 ± 50M MSL. It receives rain fall of about 300 mm per year with high humidity and temperature. The collected samples were used for further studies.

Fungi isolation

The portion of the fungi was cut, surface sterilized with 1 per cent mercuric chloride solution and then repeatedly washed with sterile distilled water [13]. The fungi were then inoculated on 2 per cent malt agar medium in petriplates. Then the fungal growth which occurred on the plates were sub cultured on malt agar slants to obtain pure culture. The samples were identified based on the morphology of the fruiting bodies and spores on the key provided previously [14, 15].

Preparation of spore suspension

The fungi were grown in malt agar medium by dissolving 20 g of malt extract in distilled water and made up to 1000 ml. The pH was maintained as 6.5 at 37°C then the plates were flooded with sterile distilled water and brushed with camel hair brush smoothly without disturbing the mycelial growth and filtered through a sterile filter. The concentration of the filtrate was adjusted to 10^5 spores/ml and inoculum was used for further studies.

Biobleaching and delignification of hard wood kraft pulp (HWKP)

The HWKP of *Eucalyptus grandis* was obtained from Tamil Nadu Newsprint and Paper industry limited karur, Tamil Nadu, India. Mycological broth (200 ml) in a conical flask (500 ml) added with a glass bead (2.5 cm dia) and HWKP (0.25%) was inoculated with fungal spore suspension (10^5 spores/ml) and incubated with shaking (200 rpm) at 25°C for 5 days. After 5 days, the resulting suspension was inoculated (15% v/v) into 500 ml flasks containing sterile water (200 ml) and 1 or 2 per cent HWKP (dry weight basis). The flasks were incubated with shaking (200 rpm) at 25°C for 2 to 10 days [16].

Mycological broth

Bactosoytone	-	10.0 g
D Glucose	-	40.0 g
*Trace element solution	-	1.0 ml
Distilled water	-	1000 ml
pH adjusted to	-	4.5 to 5.0

***Trace element solution**

$FeCl_3$	-	27.03 mg
$Na_3C_6H_5O_7$	-	24.97 mg
$CuSO_4$	-	1176.4 mg
$ZnCl_2$	-	24.97 mg
$MnSO_4$	-	476.10 mg
$MgCl_2$	-	338.02 mg
$CoCl_2$	-	118.97 mg
$NiCl_2$	-	2.377 mg
$(NH_4)_6\ Mo_7O_{24}$	-	61.80 mg
Distilled water	-	100 ml

Parameters studied

The final pH, kappa number and brightness of the treated pulp were determined. The pH of the pulp solution was measured directly by using a pH meter. Kappa number and brightness were estimated from standard hand sheets prepared from the pulp after harvest.

Preparation of hand sheet

To prepare the hand sheets (2x4 cm size), the pulp suspension was filtered through a Buchner funnel vacuum. The residue was blotted and air dried for 24 h.

Kappa number (TAPPI, 1993)

Kappa number is used as criteria for the lignin content of pulps and is determined as the volume of 0.1 N potassium permanganate (ml) consumed by 1.0 g of moisture free pulp. A portion of the cut piece of hand sheets that could consume approximately 50 per cent of potassium permanganate solution (0.1%) was weighted out and disintegrated in 500 ml distilled water until free of fibre clots or bundles. The disintegrated suspension was made up to 800 ml. To 100 ml of $KMnO_4$ solution (0.1 N), 100 ml of H_2SO_4 (4 N) was added and cooled to 25°C and immediately added to disintegrated hand sheet suspension. After 10 min, the reaction was stopped by adding 20 ml of potassium iodide solution (1 N) and titrated against sodium thiosulphate solution (0.2 N). Starch solution (0.2 %) was used as the indicator. A blank titration was carried out in the same procedure but without pulp. The kappa number was calculated by the formula

$$K = p \times f / W$$

and

$$P = (b-a)\ N / 0.1$$

Where,

K	=	Kappa number
F	=	Factor for correction to the 50 per cent permanganate consumption depending on the volume of pulp (TAPPI, 1993)
W	=	Weight of moisture free pulp sample used for estimation (g)
P	=	Amount of 0.1 N permanganate consumed by the sample (ml)
B	=	Amount of thiosulphate consumed in blank determination (ml)
A	=	Amount of thiosulphate consumed by sample
N	=	Normality of thiosulphate

Correction for reaction temperature

$$K = \frac{Pf}{W}\ [0.0+0.013(25-t)]$$

Where,

t = actual reaction temperature in degree Celsius.

Brightness

Brightness of the hand sheets were measured at 457 nm in a Perkin Elmer λ3B spectrophotometer equipped with a reflectance sphere.

RESULTS AND DISCUSSION

The results showed in table 1 and figure I, explained the treatment of HWKP by white rot fungi. The parameters were analysed for pH, kappa number and brightness (ISO units). The initial pH of the HWKP was 6.93, Kappa number was 28.00 and brightness was 31.50 ISO units. In *Trametes* sp. treatment, HWKP pH had reduced to 4.59 from 6.93 after 10 days of incubation period also the kappa number was reduced to 14.72 from 28.00, then the brightness was increased from 31.50 to 43.01 ISO Units. On the 10^{th} day of incubation period *Ganoderma* sp. reduced the pH 4.67 from 6.93, Kappa number was reduced to 13.89 on the same day, brightness was increased up to 43.98 from 31.50 ISO units. For *Poria* sp. the pH and kappa number had reduced to 4.36 and 12.76 ISO Units respectively. Increased brightness (44.87 ISO units) was observed on 10^{th} day. [10]. The pine kraft pulp was bleached in a totally chlorine free sequence that involved treatment with culture supernatant from white rot fungus *Trametes troggi* where as kraft pulp from *Eucalyptus globules* was treated by *Pycnoporus sanguienus* at 40°C on pH 3.0 [17]. *T. versicolor* was shown to be capable of substantial depolarization and delignification of unbleached industrial kraft pulps over 2 to 5 days [18].

Table 1: Biobleaching and delignification of hardwood kraft pulp (HWKP) by white rot fungi

Incubation period (days)	Final pH			Kappa number			Brightness (ISO units)		
Control	Tr	Gn	Pr	Tr	Gn	Pr	Tr	Gn	Pr
0	6.93	6.93	6.93	28.00	28.00	28.00	31.50	31.50	31.50
2	6.39	6.42	6.01	26.80	26.20	24.93	34.98	35.01	35.08
4	6.15	6.29	5.93	24.30	23.50	21.90	38.14	38.96	39.21
6	5.92	5.84	5.23	20.34	19.89	19.23	40.26	41.13	42.17
8	5.39	5.42	5.12	17.39	16.92	15.34	42.38	43.26	44.18
10	4.59	4.67	4.36	14.72	13.89	12.76	43.01	43.98	44.87

Tr: *Trametes* sp.; Gn: *Ganoderma* sp.; Pr: *Poria* sp Values are mean of three replicates

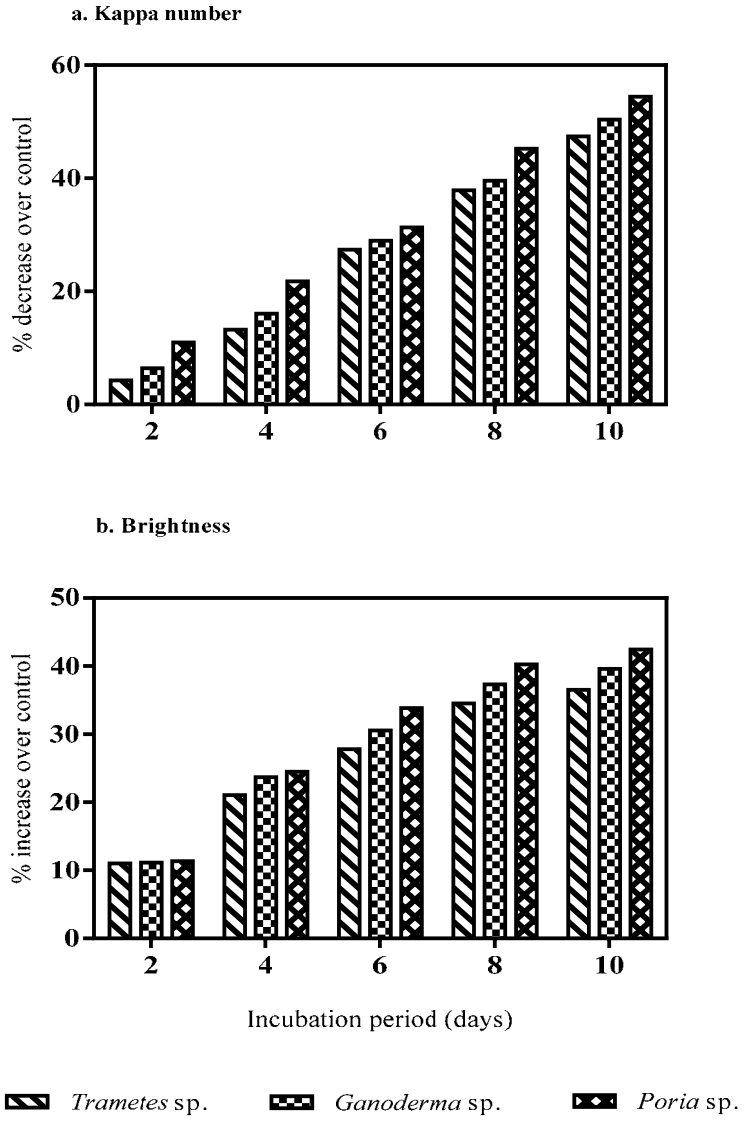

Fig. I: Effect of fungal treatment on kappa number and brightness of HWKP

CONCLUSION

Biobleaching and delignification of HWKP, all the three white rot fungi reduced the kappa number (12.76%) and increased the brightness (44.87%) of the pulp on 10 days of incubation. But, the maximum reduction of kappa number and increased the brightness were noted in *Poria* sp.

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