Research Article

Biobleaching and Delignification of Hard Wood Kraft Pulp by White Rot Fungi

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ABSTRACT
In the present study lignin degrading white rot fungi, *Schizophyllum commune* and *Lenzites eximia* were collected from the living tree of *Tamarandus indica* and burnt tree respectively from the Western Ghats region of Tamil Nadu, India. The collected fungi were isolated and identified based on the key provided previously (Bakshi 1971; Gilbertson and Ryvarden 1986). *Phanerochaete chrysosporium* 787 was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and was used as the reference fungus. The selected fungi were tried for biobleaching and delignification of hard word kraft pulp. In biobleaching and delignification of HWKP, all the three fungi reduced the kappa number and increased the brightness of the pulp appreciably after five days incubation. The maximum reduction of pH (3.26) was found in *L. eximia*. Maximum reduction in kappa number (47.24 %) and brightness (45.89 %) was noted in *L. eximia.*

Keywords: Biobleaching, *Schizophyllum commune*, *Lenzites eximia,* Hardwood Kraft Pulp.

INTRODUCTION

Pulp is a lignocellulosic fibrous material prepared by chemically, mechanically or biologically separating cellulose fibers from wood. Wood pulp is the most common raw material in paper making. The production of pulp and paper involves three major processing steps that include pulping, bleaching and paper production. The purpose of bleaching is to remove the residual lignin and to brighten the pulp. The type of pulping and the amount of bleaching used depends on the nature of feedstock and the desired quality of the end product. Multistage bleaching procedures using a combination of treatment with chlorine based chemicals and alkaline extraction are normally used in bleaching of kraft pulp. There has been growing environmental concern about chlorinated organic substances, including toxic, mutagenic, carcinogenic polychlorinated dioxins, dibenzofurans and phenols in the effluent from the bleaching of conventional kraft pulp (Alder *et al.,* 2009). Fungal treatment helps to decrease the negative environmental impact of pulp and paper production. Brightness is a measure of light that is reflected by paper under specified conditions and is usually reported in percentage, so a higher number represents a brighter or whiter paper. The use of lignin degrading fungal cultures and their lignin degrading enzymes in the pulp and paper industry has been intensively studied and commercial applications have been developed. The advantages of biobleaching includes reduced consumption of bleaching chemical; reduced adsorbable organic halogen; improved pulp and paper quality; improved brightness; reduced effluent toxicity and pollution load. White rot fungi produce extracellular oxidative enzymes, which initiate oxidation of lignin. Due to their lignin degrading capacity, whole cultures of various white rot fungi cause extensive brightness gains and delignification of kraft pulp (Scott *et al.,* 2000).

Lignocellulose is the predominant component of woody plant and dead plant materials, and the most abundant biomass on earth. Removal of lignin from wood is the first step in the manufacturing of chemical paper pulps and the most common process (Re *et al.*, 2010). 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. It has been reported that chlorinated products derived from lignin

during these bleaching procedures are mutagenic (Ander, 1977). 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 lead 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 (Mirsha *et al.,* 2001). 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 prebleaching. Bioleaching has number of advantages such as reduction of AOX levels in discarded effluents and improved pulp quality gain in brightness (Thakur *et al*., 2012). Pretreatment of wood chips with proper fungi results in significant energy and chemical savings and allows for an improved paper quality (Moreira *et al.,* 2001). The importance of microbial enzymes in pulp and paper manufacturing has grown significantly in the last two decades (Re *et al.,* 2008). White rot fungi can degrade lignin and a range of environmental pollutants by many of their extra cellular lignolytic 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 (Lawson and Still, 1957). Kirk and Yang (1979) were the first to recognize that *P. chrysosporium* could partially delignify softwood 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 (Paice *et al*., 1989). Islam *et al*. (2008) reported the beneficial effects of bleaching by the white rot fungi *Ceriporiopsis subvermispora, P. chrysosporium* and *Trametes (Coriolus) versicolor.*

MATERIALS AND METHODS

Collection of fungi

The fungi *Schizophyllum commune* and *Lenzites eximia* were collected from Western Ghats area of Tamilnadu, India and were isolated from living tree of *Tamarindus indica,* burnt tree respectively. The collection sight was situated in the latitude of -11.58,^{o}S and longitude of 76.93^{o}E at 400 ± 50M MSL. It receives rain fall of about 300 mm per year with high humidity and temperature. The reference fungus *Phanerochaete chrysosporium* 787 was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and was used for further studies.

Isolation of the fungi

The portion of the fungi was cut, surface sterilized with 1 per cent mercuric chloride solution and then repeatedly washed with sterile distilled water (Roy Watling, 1971). The fungi were then inoculated on 2 per cent malt agar medium in petriplates. Then the fungal growth which occurred on the plates was sub cultured on malt agar slants to obtain pure culture. The samples were identified based on the morphology of the fruiting bodies and spores based on the key provided previously by (Bakshi 1971; Gilbertson and Ryvarden 1986).

Preparation of spore suspension

The fungi were grown in malt agar medium by dissolving 20 g of malt extract and 20 g of agar in distilled water and made up to 1000 ml. The pH was maintained as 6.5 at 37^{o}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 used as inoculums for further studies.

Biobleaching and delignification of hard wood kraft pulp (HWKP) by white rot fungi

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 5 days (Archibald *et al.,* 1990).

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 manner 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 p (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

Biobleaching and delignification of hardwood kraft pulp (HWKP)

The results presented in Table 1 and figure I, revealed that the efficiency of white rot fungi in bleaching and delignification of HWKP and it had an initial pH of 7.00; the kappa number was 29.0 and brightness was 22.40 ISO units. The treatment of HWKP by *P. chrysosporium* 787 after five days incubation period lowered

the pH to 3.64 and also reduced the kappa number by 43.1 per cent; the brightness increased by 34.6 per cent.

In *S. commune* treatment of HWKP pH has reduced to 3.86 after 5 days of incubation period and also reduced the kappa number by 44.48 per cent; the brightness was increased to 39.73 per cent.

When the HWKP was treated with *L. eximia*, the pH had reduced to 3.26 after five days whereas maximum reduction of kappa number was observed to be 47.24 and the increase in brightness was found to be 45.89 per cent.

Multistage bleaching process consisting of chlorination and alkaline extraction stages are are conventionally used in the bleaching of kraft pulp and the effluents from these processes are toxice and mutagenic. Bioleaching with white rot fungi has been studied to establish chlorine free bleaching process (Miura *et al.,* 1998). Tim *et al.* (2001) established that cellobiose dehydrogenase (CDH) deficient strains of the basidiomycete *T. versicolor* were produced by transforming protoplasts of strain 52J with a plasmid carrying the *T. versicolor* CDH gene. It biobleached and delignified industrial unbleached kraft pulp as efficiently as wild type *T. versicolor*, indicating that CHD is not required for the degradation and biobleaching of kraft lignin. Jian *et al*. (2006) reported that, modification of bleached pulp with enzymes of 41.0 U/g (on oxylanase) form *Asperigillus* L22 decrease pulp kappa number by 6.29 and 12.07 per cent and increased pulp brightness. Veronica *et al*. (2010) examined that loblolly pine kraft pulp was bleached in a totally chlorine free sequence that involved treatment with culture supernatants from white rot fungus *Trametes troggi* followed by a peroxide stage. *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 per cent (Arbeola *et al*., 1992).

CONCLUSION

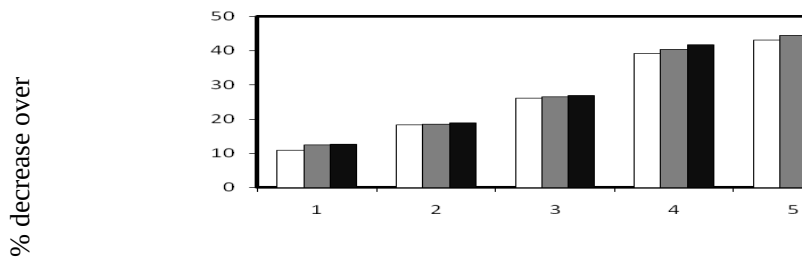
In biobleaching and delignification of HWKP, all the three fungi reduced the kappa number and increased the brightness of the pulp appreciably after five days incubation. The maximum reduction of pH (3.26) was found in *L. eximia.* Maximum reduction in kappa number (47.24 %) and brightness (45.89 %) was noted in *L. eximia.*

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

Incubation period (days)	Final pH			Kappa number			Brightness (ISO units)		
	Pc	Sc	Le	Pc	Sc	Le	Pc	Sc	Le
0	7.00	7.00	7.00	29.0	29.0	29.0	22.40	22.40	22.40
1	6.05	6.52	5.43	25.8	25.4	25.3	24.32	25.07	25.13
2	5.29	5.85	5.03	23.7	23.6	23.5	26.11	26.79	27.03
3	4.23	4.73	4.01	21.4	21.3	21.2	27.03	27.79	28.54
4	3.92	4.07	3.60	17.6	17.3	16.9	28.29	29.36	30.15
5	3.64	3.86	3.26	16.5	16.1	15.3	30.17	31.30	32.68

Pc: *Phanerochaete chrysosporium* 787; Sc: *Schizophyllum commune*; Le: *Lenzites eximia*
The values are mean of three replicates

a. Kappa number



b. Brightness

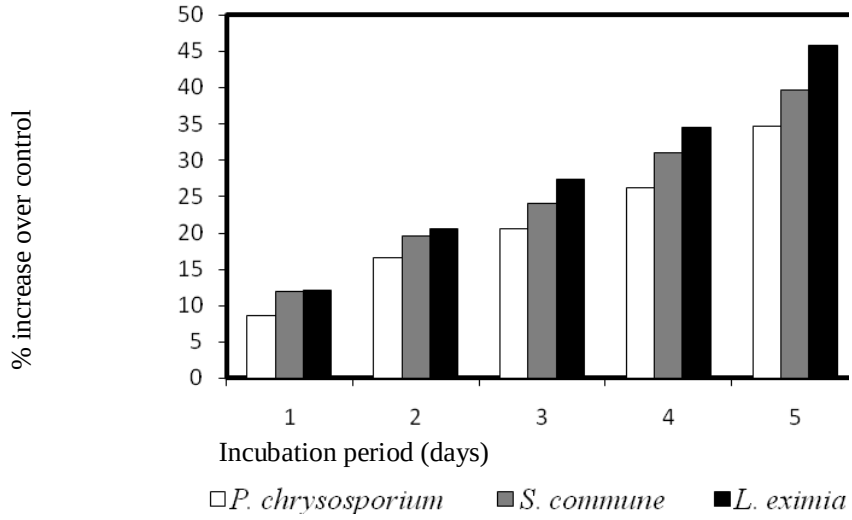


Fig. 1 : Effect of fungal treatment on kappa number and brightness of HWKP

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