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DECOLOURISATION OF RAYON EFFLUENTS AND BLEACHING OF KRAFT PULP BY *STEREUM OSTREA*

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ABSTRACT

A potential lignolytic fungus, *Stereum ostrea* was analysed for the decolourisation of industrial effluents discharged from rayon pulp mills. The colour removal was from 5580.85 to 2690.05 CU during 40 days of incubation time. The laccase activity during the decolorization process was increased up to 20th day (862.77 U/L) and after that it was very low. Chemical bleaching was effective and showed 92-94% of brightness with viscosity 14 cp, while biobleaching of pulp recorded 78% of brightness with 4 cp viscosity during 40 days of solid state fermentation. The rayon sheet, after biobleaching was prepared and 76% brightness and 8 cp viscosity was noticed, which was accepted with moderate response in industries.

KEY WORDS

Decolorization, *Stereum ostrea*, kraft pulp, rayon effluents, biobleaching.

INTRODUCTION

Billions of gallons of toxic and intensely colored waste effluents are released into the rivers and streams annually by the pulp and paper industries[1]. The primary contributor to the colour and toxicity of these aquatic systems is the pulp bleach plant effluents (BPE), which contains largely high molecular weight, modified and chlorinated lignin and its degradation products[2, 3, 4, 5]. These toxic effluents are generated during paper and rayon manufacturing process and also in the bleaching of pulp with conventional chemical methods in different stages. The bleaching agents used in pulp and paper industries are chlorine, alkali, hypochlorite and hydrogen peroxide. The use of chlorine based chemicals in the bleaching process generate chloro-lignin or chloro phenol compounds which are completely resistant to microbial attack and remain as recalcitrants[6, 7]. Therefore, environmental concerns have led us to seek alternative ways to eliminate or at least reduce, the use of chlorine-based chemicals in bleaching of pulp. The current biotechnological approach is concerned to tackle this problem. The two phased solutions are being developed; one is biobleaching of the pulp[8, 9] which can be used as alternate method for chemical bleaching to avoid the generation of toxic effluents and the second is biotreatment of bleach plant effluents[10] for their decolourisation and dechlorination.

White rot basidiomycete fungi are the only known organisms which are capable of degrading lignin by releasing lignolytic enzymes such as laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP)[11, 12, 13, 14]. Ligninolytic fungi, *Phanerochaete chrysosporium* and *Trametes versicolor* can efficiently decolourise and dechlorinate BPE[15, 16 17, 18, 19, 20]. Because of the importance of lignin as a renewable source for the production of paper, feeds, chemicals and fuels, there has been an increasing research emphasis on the fungal degradation of lignin. Delignification of lignocellulosic materials by white rot fungi is of great interest and has been investigated to improve the digestibility of wood or straw for animal feed[21, 22, 23, 24, 25]. Lignin biodegradation by *Phanerochaete chrysosporium* and *Trametes versicolor* has been intensively studied to delignify and brighten unbleached kraft pulp (UKP) [26, 27, 28]. Better lignin-degrading systems were identified in different fungi for use in various biotechnological applications[29, 30, 31, 32].

In view of the above findings, a potential lignolytic fungus, *Stereum ostrea* was analysed for the decolurisation and biobleaching of bleach plant effluents and unbleached kraft pulp of rayon industries. The medium used in this experiment was optimized for maximum laccase production, comparing to other white rot fungi, *Phanerochaete chrysosporium, Coriolus versicolor* or *Phlebia radiata*.

MATERIAL AND METHODS

Collection of basidiomycete:

The fruit bodies of *Stereum ostrea* were collected in rainy season from the surroundings of Warangal. The fruit bodies were preserved by sealing in polythene bags and noted in our departmental fungal herbarium.

Identification of white rot fungi:

Fungus was identified based on the fruit body characters and spore print[33, 34, 35, 36].

Culturing of fungal strain:

A small piece of fruiting body was dipped in 0.01% mercuric chloride to remove the surface contamination and washed several times with distilled water to remove the traces of $HgCl_2$

and transferred aseptically onto 3% malt agar slants. Slants were incubated for 5 to 7 days and initial growth of mycelium was observed on 5th day.

Maintenance of fungi:

The mycelium collected from the growing edge was transferred onto new malt agar slant and incubated further 5 to 7 days and slants were stored at 4°C until use. The culture was sub cultured at an interval of 30 days before being utilized as an inoculum.

Decolorisation studies:

The liquid and solid-state fermentation systems were followed in the studies on decolourisation of effluents and bleaching of pulp.

Liquid fermentation system:

Primary out let effluents released from the pulp bleaching plant of A.P. Rayon factory was used for the liquid cultures. This effluent released from the different bleaching stages contains pulp in diluted concentration and is used for secondary recovery of the pulp. Malt medium containing 33.3% of this effluent (control medium) supplemented with Cu^{++} (2 mgL^{-1}) was used as liquid culture. Each 250 mL of flask containing 100 mL medium was inoculated with three pieces of 2 mm^2 fresh fungal mycelium from ten days precultured malt agar plates. An uninoculated medium served as blank. Decolourisation was observed up to 40 days. Culture harvests of different days were filtered and filtrates were centrifuged for 15 min. at 3000 rpm for 30 min. and reduction in colour was read at 465 nm with reference blank. Colour units were calculated[37] by using conversion factor 4,015 standard platinum cobalt colour units per A_{465} of 1.0.

Solid state fermentation system:

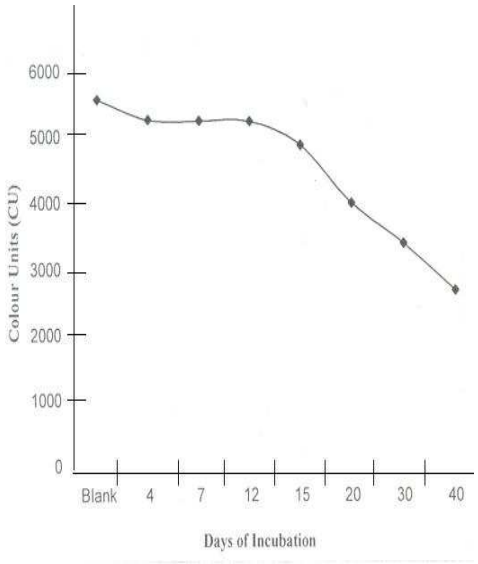
Biobleaching of the pulp in the solid-state fermentation system was followed[38]. Fifteen mL of M2 medium supplemented with Cu^{++} (2 mgL^{-1}) was added to each 250 mL conical flask containing 50 g of unbleached cooked pulp (brightness 45%, viscosity). Flasks were sterilized by autoclaving at 10 lbs pressure for 30 min. Flasks were inoculated with 2 mm^2 of fresh fungal mycelium from 10 days precultured malt agar plates. An uninoculated flask containing pulp was served as blank. Rate of decolourization was measured in terms of increase in brightness. After incubation with fungi, pulp samples were washed with water and pulp sheets were prepared with a porceline Buchner funnel (diameter, 11 mm) and air dried. Brightness was determined with Aimil glass reflectance meter (Cat.No.070, S.No.95358). Viscosity of the pulp was measured by the method developed by department of quality control, A.P. Rayon factory, using capillary viscometer. 0.255 g of pulp was dissolved in 25 mL of cuprammonium solution and 2-3 drops of pyrogalla (1:3 diluted) was added and stirred for 15 min. on magnetic stirrer. This solution was used to measure the flow time of the solution. The flow time was converted into centiopoises (cp) using standard chart.

RESULT AND DISCUSSION

Decolourisation of effluent was studied in liquid and solid cultures up to 40 days. In liquid cultures of *Stereum ostrea* the colour reduced from 5580.85 to 2690.05 CU (Fig.1). Decolurisation was 5.6% in 4th day cultures and, the reduction in colour was very less up to 12th day. On 40th day of incubation the percentage of decoularisation was maximum (50%).

Fig. 1

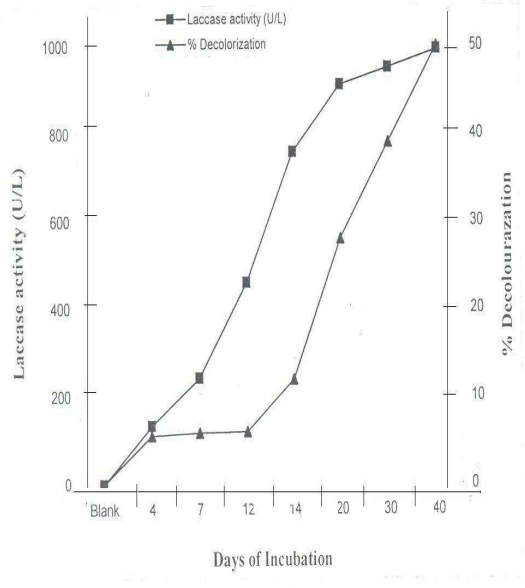
Decolorisation of bleachery effluents by Stereum ostrea in different days of incubation



In the comparison between laccase production and decolourisation, it was noticed that the laccase activity in culture filtrates was gradually increased up to 20th day of incubation (862.77 U/L) and the raise in laccase activity was very low between 20th and 40th day of incubation. The increase in laccase activity within 20 days of incubation was 86.94% and between 20th to 40th day it was 13% (Fig. 2).

Fig. 2

***Correlation between laccase activity and decolorisation by Stereum ostrea in different days of* incubation**



The relationships between brightness of the pulp and viscosity changes during chemical bleaching were studied and recorded (Table 1a). By chemical bleaching the percent of brightness was gradually increased (45-94%) and at the same time there was a reduction in the viscosity. The viscosity should be optimum in the pulp to prepare and strengthen the quality of rayon sheets. In the final step of chemical treatment, the brightness of the rayon sheet was 92-94% and viscosity was 14 cp, which was ideal for industrial rayon sheet synthesis.

Table 1

a) Brightness and viscosity changes during chemical bleaching of kraft pulp

Bleaching stage	Brightness (%)	Viscosity (in centipoises)
Unbleached pulp	45	35
Chlorination stage (Cl_2)	55	28
Oxygenated H_2O_2 stage (EOP)	68	21
Hypo I stage	85	18
ClO_2	89	16
Extracted peroxidase stage (EP)	90	15
Rayon sheet	92-94	14

The correlation between brightness of the pulp and its viscosity status during solid state fermentation (SSF) with *S.ostrea* was recorded (Table 1b). The brightness of the pulp was improved from 45-78%, while viscosity loss was 35-4.4 cp. Though the manufacture of the rayon sheet was possible with this viscosity, the sheet strength and quality were not up to the mark. This is due to utilization of cellulose by the growing organism which converts the cellulose to sugars for its easy carbon assimilation.

Table 1

b) Brightness and viscosity changes during biobleaching by Stereum ostrea during solid state fermentation of kraft pulp

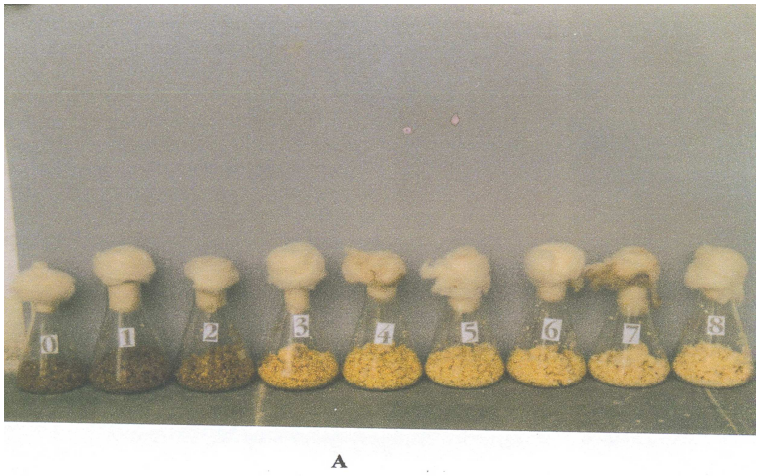
Days of incubation	Brightness (%)	Viscosity (in centipoises)
Blank	45	35
4	49	23.4
7	54	11.8
12	71	9.2
15	73	8.8
20	76	8
25	78	6.2
30	78	5.5
40	78	4.4

The decolourization of pulp by *S.ostrea* during 40 days of SSF was presented in plate 1A. The activity of the organism to utilize the lignin and initiation of brightness was started after 7 days of incubation period and gradually it reached to 78% by 40 days. A comparison in the rayon sheet prepared with unbleached kraft pulp and biobleached pulp after 20days of incubation was presented in plate 1B. In this biobleached rayon sheet the brightness percent was 76 and viscosity was 8 cp. This was considered as moderately acceptable process of pulp for the rayon preparation. Here, the critical point is improvement of brightness of the pulp without causing any damage to the cellulose,

Though the process was viewed as cost-effective (by comparison with chemicals), renewable and eco-friendly, care must be taken to maintain the viscosity of the pulp for quality rayon sheet. For this purpose the *S.ostrea* was subjected to mutations by ultra violet radiations for the development of cellulose minus strains (data not presented).

Plate 1A

Decolourisataion of kraft pulp by Stereum ostrea in different days of incubation (50 g of pulp + 15 mL of M2 medium)

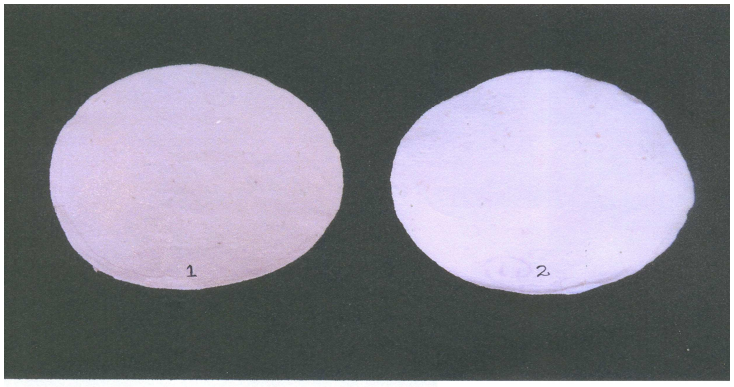


A

0: Blank; 1: Four days; 2: Seven days; 3: Twelve days; 4: Fifteen days; 5: Twenty days; 6: Twenty five days; 7: Thirty days; 8: Forty days.

Plate 1B

Comparison between unbleached (1) and bleached (2) pulp sheet with Stereum ostrea after twenty days of incubation



B

A number of previous studies[20, 39, 40,] have shown that *Phanerochaete chrysosporium* as well as some other white rot fungi rapidly decolourise BPE. BPE from first alkali extraction stage after chlorination was decomposed to low molecular –weight colourless products[16]. *Phanerocheate chrysosporium* was immobilized[2,17] on partially submerged rotating disks to decolourise industrial BPE. The role of lignin peroxidase and manganese peroxidase activities were not reported in any of these previous studies on decolourisation or degradation of lignin based effluents. The present study is aimed at the delignification and decoloruisation of rayon effluent and unbleached kraft pulp by ligninolytic enzymes produced by *Stereum ostrea*. The culture conditions were optimized for the maximum production of laccase. The results indicated that the *Stereum ostrea* can decolorize the diluted effluents up to 50% in 40 days of incubation. But the laccase activity in this culture was increased up to 20 days of incubation but remained almost constant after 20th day. This suggests that the decolourisation and laccase production are not happening simultaneously. These results are comparable with the reports available on decolourisation of kraft bleach plant effluent[41, 42, 43, 44].

Effluent cultures were used with final concentration of 3000 CU and achieved 85% decolourisation with lignin peroxidase and manganese peroxidase produced by *Phanerochaete chrysosporium*[37]. Ninety percent was achieved declourisation in CEH effluent with *Stagnospora gigaspora*, a coelomycetous fungi[10]. *Phanerochaete chrysosporium* was used for biobleaching in liquid-state fermentation system and reported that kappa number was decreased by 60% after a subsequent alkali treatment[45]. *Trametes versicolor* in liquid-state fermentation system achieved 15 point increase in brightness[46]. The same level of cumulative manganese peroxidase production by *Phanerochaete chrysosporium* and *Trametes versicolor* in the solid-state and liquid-state fermentation systems, but observed negligible increase in brightness in the liquid-state fermentation system[40].

Most studies on the biobleaching of UKP by *Phanerochaete chrysosporium* and *Trametes versicolor* were carried out in the liquid-state fermentation systems with 15% pulp consistencies[39,45]. On the other hand biobleaching of the unbleached kraft pulp in the solid-state fermentation afforded a marked brightness increase[40,46]. The sequential treatment of softwood kraft pulps with laccase resulted in lower kappa number and higher brightness[47]. Therefore, the present study aimed at biobleaching of unbleached kraft pulp by *Stereum ostrea* in the solid state fermentation system with little supplementation of nitrogen limited medium with Cu^{++} ions (2 mgL^{-1}). It was recorded that maximum increase in brightness was in the solid-state conditions. These results supported the concept of effective lignin-degradation under solid-state fermentation by white-rot fungi[48, 49]. The results further clarified that the biobleaching process can significantly increase the brightness of the pulp and reduce the use of chlorine based chemicals and pollution load of waste liquor. The white rot fungus, IZU-154, degraded wood lignin more extensively and selectively than *Phanerochaete chrysosporium* and *Trametes versicolor*[50]. In addition, the use of chlorine based chemicals were reduced in bleaching of UKP in the biobleaching process, which combined a IZU-154 treatment and chemical bleaching[51, 52, 53].

A solid state bioreactor design for application in industrial process was attempted[54]. The viscosity of the pulp is the crucial factor in the preparation of rayon sheets, which dropped substantially during solid state fermentation by *Stereum ostrea*. This may be due to the presence of cellulose enzyme which are acting on the cellulose fibrils of the pulp and reducing the viscosity. The enzyme production, brightness and viscosity losses are not in a line during the delignification of kraft pulp by *Stereum ostrea* in different incubation periods. The correlation

between degradation of lignin with effluent decolourization by white rot fungi was studied, where they used the black liquor and pulp effluents to observe the colour removal[55]. The decolorization of triaryl methane, ingold, azo and anthraquinone dyes by *Trametes hirsute* was recorded[56]. The waste water of textile industry has been decolorized efficiently by white rot fungus, *Irpex lacteus* without adding any chemicals and recorded[57] the decolourization of dye effluents by shaking (59%) and stationery cultures (93%).

The pulp bleaching in chlorine free sequence using laccase mediator system of *Pycnoporus cinnabarinus, Trametes versicolor and Pleurotus eryngii* and recorded[58] best preservation of cellulose and the largest removal of residual lignin content. The degradation and decolourization of synthetic effluents consisting of mixture of ten dyes by using *Trametes villosa* and *Pycnoporous sanguineus* was found the potentiality of these fungi for environmental decontamination[59]. The treatment of the effluents from kraft bleach plant with *Pleurotus sajor-caju* and *P.ostreatus* and recorded the decolurization by 57and 76 percent respectively after 14 days of incubation[60]. The extracellular enzyme activities and ability to degrade the synthetic dyes of a white rot fungus, *Gramnothele subargentea* was reported that this organism is a useful tool in the pulp industry and bioremediation[61]. The biological decolourization of Cibacron black W-NN using five commercial *Pleurotus* species was reported among which *P. sajor-caju* fully decourized the dye[62]. The industrial and biotechnological applications of lignolytic enzymes of the basidiomycete members were studied to replace the conventional chemical processes of several industries[63]. The colour reduction of paper industry effluents by white rot fungus, *Sporotrichum pulverulentum* was showed that aeration and sterilization played an important role in the colour removal[64].

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