BIOBLEACHING AND DECHLORINATION OF PAPER PULP USING FUNGAL CULTURES -A BIOREMEDIATION APPROACH

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Abstract

To replace or substitute the conventional pulp bleaching chemical chlorine, bio enzymes from fungi were utilized in this study. Thus, environmental contamination by formation of organochlorines, in the downstream could be controlled in the process stage itself. The enzymatic activities and bleaching efficiencies of kraft pulps obtained from various stages of chemical bleaching by fungal enzymes are discussed hereunder. The kappa number reduction (bio bleaching) of the thickener stage pulp was 18.8% with *Phanerochaete chrysosporium* and 9.09% with *Trametes versicolor* compared to the uninoculated control. But for the chlorine stage pulp, *Trametes versicolor* brought about 4.54% reduction of kappa number while there was no reduction in kappa number under *Phanerochaete chrysosporium.* In case of the EP stage pulp, the enzymatic extracts of *P. chrysosporium* brought about 31.3% reduction and *T. versicolor* contributed to 25.0% reduction in kappa number. With reference to the Hypo stage pulp also, *P. chrysosporium* brought about maximum reduction of 33.3% compared to *T. versicolor* (22.2% reduction).

Key words

Kappa number, *Trametes versicolor, Phanerochaete chrysosporium,* Biobleaching.

Introduction

Certain white-rot fungi like *Trametes versicolor, Phanerochaete chrysosporium* could reduce the lignin content and increase the brightness of kraft pulps without minimizing the pulp quality and quantity. The enzymatic delignification effect of these white rot fungi can be utilized in the bleaching sequences of the pulp mill. Direct physical contact between the fungal hyphae and the pulp fibers is not required, but the presence and enzymatic activity of the live fungus is necessary for long-term delignification.

Methodology

White rot fungal cultures of *Phanerochaete chrysosporium* and *Trametes versicolor* from Tamil Nadu Agricultural University culture collection were utilized for the study. The 5 day old fungal cultures were inoculated in the mineral salts media with nitrogen content of 0.2% and 1% and incubated in shaker of 250 rpm at 25-30°C. To prevent the fungal pellet formation glass marbles were added to the culture media and fragmented hyphal suspension was obtained. (Kirkpatrick *et al.,* 1989).

Treatment details

Factor I - **Fungal cultures**

F_1 - *Phanerochaete chrysosporium*

F_2 - *Trametes versicolor*

Factor II - **Dosage – Concentration of fungal liquid suspension cultures and Nitrogen contents**

D_1 - Pulp

D_2 - Pulp + fungal suspension undiluted [Nitrogen 0.2 %]

D_3 - Pulp + fungal suspension 2 fold diluted [Nitrogen 0.2 %]

D_4 - Pulp + fungal suspension 4 fold diluted [Nitrogen 0.2 %]

D_5 - Pulp + fungal suspension undiluted [Nitrogen 1 %]

D_6 - Pulp + fungal suspension 2 fold diluted [Nitrogen 1 %]

D_7 - Pulp + fungal suspension 4 fold diluted [Nitrogen 1 %]

Factor III - **Pulp substrate**

S_1 - Thickener stage pulp

S_2 - Chlorine pulp

S_3 - EP pulp

S_4 - Hypo pulp

100 g of autoclaved pulp was taken in polypropylene bags. 10 ml of fungal culture suspensions were inoculated into the pulp samples according to the treatment details and incubated at room temperature Pulp samples were taken on the tenth day of incubation and analyzed for kappa number, lignin concentration and residual chlorine.

Estimation of kappa number

The kappa number is the volume of 0.1 N potassium permanganate solution utilized by one gram of oven dry pulp under the specified conditions.

One gram of oven dry pulp was mixed with 500 ml water and crushed until it was free of fibre clots. Disintegrated sample was transferred to a 2000 ml beaker and volume made up to 795 ml with distilled water (25 ± 0.2°C). 100 ml of 0.1 N $KMnO_4$ and 100 ml of 4 N H_2SO_4 were pipetted out and volume made up to 1000 ± 5 ml. After 10 minutes the reaction was ended by adding 20 ml of 10% KI solution. Using starch indicator, the solution was titrated against sodium thiosulfate. Simultaneously blank titration was carried out without the pulp.

$$K = P\,x\,f\,/W\,, P = ((b-a))/0.1\,x\,N$$

Where,

K = Kappa number

f = factor for correction to a 50% $KMnO_4$

W = Wt. of moisture free pulp
P = amount of 0.1N $KMnO_4$, actually consumed by the sample (ml)
b = amount of thiosulfate consumed by the blank (ml)
a = amount of thiosulfate consumed by the sample (ml)
N = Normality of thiosulfate

(TAAPI Standard T236,1976)

Estimation of residual chlorine

To 100 ml of pulp supernatant ,10 ml of 10% acetic acid and 10 ml of 10% KI were added and titrated against 0.2N $Na_2S_2O_3$ using starch indicator.

Results

Kappa number

Thickener pulp

The fungal culture F_1 (*Phanerochaete chrysosporium*) registered the lowest kappa number (19.1) followed by F_2 (*Trametes versicolor*) (20.1).

Among the dilution treatments, D_2 (0.2% N, undiluted) recorded the lowest (18) kappa number and D_1 (control) registered the highest (22) kappa number.

The interaction between the cultures and dilution treatments showed that F_1D_2 (undiluted culture of *P.c* at 0.2% N level) registered the lowest kappa number of 17.5 followed by F_1D_3 (2 fold diluted culture of *P.c* at 0.2% N level), F_1D_4 (4 fold diluted culture of *P.c* at 0.2% N level) and F_2D_2 (undiluted culture of *T.v* at 1% N level) and all the above were on par with each other.

Chlorine stage pulp

While considering the fungal cultures, F_2 (*Trametes versicolor*) recorded the lowest kappa number of 18.8 followed by F_1 (*Phanerochaete chrysosporium*) of 19.7 kappa number and both were significantly different from the other.

With regard to the dilutions of suspension cultures, D_2 (undiluted at 0.2% N) registered the lowest kappa number, followed by D_3 (2 fold diluted at 0.2% N) and D_5 (undiluted, 1% N) and they were on par with each other.

EP stage pulp

Among the white rot fungal cultures, F_1 (*Phanerochaete chrysosporium*) recorded the lowest kappa number of 11.5 and F_2 (*Trametes vericolor*) registered the highest kappa number (12.7).

The interaction between, fungal cultures and dilutions of suspension cultures revealed that, F_1D_2 (undiluted suspension culture of *P. chrysosporium*) recorded the lowest kappa number of 9.5 which was significantly different from others. The interaction between days of incubation showed that F_1 (*P. chrysosporium*) on the 10^{th} day recorded the lowest kappa number and was significantly superior to the rest.

Hypo stage pulp

Regarding hypo stage pulp, the lowest kappa number of 6.2 was noticed after 10 days of incubation and the highest of 7.4 on the 5^{th} day.

The culture F_1 (*Phanerochaete chrysosporium*) recorded the lowest kappa number of 6.2 and was significantly different than F_2 (*Trametes versicolor*) with kappa number of 7.4.

Regarding the interactions, culture F_1 (*P. Chrysosporium*) under D_2 (undilution, 0.2% N level) recorded the lowest kappa number of 4.0 and was significantly superior to F_1D_1 (control) with kappa number of 9.0.

Table 1 Influence of white rot fungal extracts and Nitrogen nutrition on the kappa number of the paper pulp

Treatments	S1		S2		S3		S4	
	F1	F2	F1	F2	F1	F2	F1	F2
D_1	22	22	16	16	20	20	9	9
D_2	16	18	9	10	19	19	6	5
D_3	17	19	9	10	20	19	7	6
D_4	17	20	10	12	20	20	7	7
D_5	18	19	10	12	20	19	7	6
D_6	19	20	11	13	20	19	8	7
D_7	19	20	11	14	20	20	8	8
Mean	**18**	**20**	**11**	**12**	**20**	**19**	**7**	**7**

Residual chlorine content of pulps of various stages after the incubation period

Chlorine stage

At the end of experimental period, the residual chlorine content of the pulp was the highest of 48 % under D_1 (control) followed by 46.5 % in D_7 (4 fold dilution at 1% N level) and both were on par with each other.

Among the two cultures F_1 (*Phanerochaete chrysosporium*) registered the highest residual chlorine content of 45.4 % followed by F_2 (*Trametes versicolor*) of 43.6

EP stage pulp

Among the cultures F_1 (*Phanerochaete chrysosporium*) recorded the highest residual chlorine content of 846.2 ppm and the lowest value of 658.4 ppm was recorded under F_2 (*Trametes versicolor*).

With respect to treatments, D_1 (control) recorded the highest residual chlorine content of 950 ppm. The lowest value of 650 ppm was recorded by D_7 (1% N, 4 fold dilution).

Regarding the interaction between cultures and dilutions, the highest residual chlorine content of 950 ppm was recorded in F_1D_1 (*Phanerochaete chrysosporium*, control) and F_2D_1 (*T. versicolor*, control). The lowest residual chlorine content 550 ppm was observed in F_2D_2 (*T. versicolor*, 0.2% N in undiluted culture suspension).

Hypo stage pulp

In the hypo stage pulp, the maximum residual chlorine content of 44.5 ppm observed under F_1 (*Phanerochaete chrysosporium*) and the maximum residual chlorine content of 42.3 ppm was registered under F_2 (*Trametes versicolor*).

Among the dilutions of suspension cultures, D_1 (control) recorded the highest residual chlorine content (47 ppm). D_2 (0.2% N undiluted suspension) recorded the lowest residual chlorine content of 40.5 ppm followed by D_5 (1% N, undiluted suspension) and both were on par with each other.

Table 2 Residual chloride concentration of pulps of different stages as influenced by white rot fungal extracts

Treatments	S2 (%)			S3 (ppm)			S4 (ppm)		
	F1	F2	Mean	F1	F2	Mean	F1	F2	Mean
D_1	48	48	**48**	950	950	**950**	47	47	**47**
D_2	43	39	**41**	750	550	**650**	42	38	**40**
D_3	44	43	**44**	783	588	**686**	44	41	**43**
D_4	44	44	**44**	840	630	**735**	46	43	**45**
D_5	45	41	**43**	835	590	**713**	42	39	**41**
D_6	47	44	**46**	856	623	**740**	44	43	**44**
D_7	47	46	**47**	910	678	**794**	47	45	**46**
Mean	**45**	**44**	**45**	**846**	**658**	**752**	**45**	**42**	**43**

The supernatant enzymatic extract of the *Trametes versicolor* culture was more effective than that of the other white-rot fungi examined with respect to dechlorination of bleached kraft pulp at EP stage of the bleaching process. This could be due to the fact that the laccase enzyme from *Trametes versicolor* uses oxygen as an electron acceptor during laccase dependent oxidation reactions. The higher amount of dissolved oxygen present in the EP stage pulp favoured dechlorination (Kirk *et al*., 1987; Yarapolov *et al*.,1994). This is in agreement with the findings of Unal and Kolonkaya (2001) according to whom, the dechlorination activity of *Trametes versicolor* was more than that of the other fungi examined.

Fig 1 Bleaching of Thickener pulp using *Phanerochaete chrysosporium*

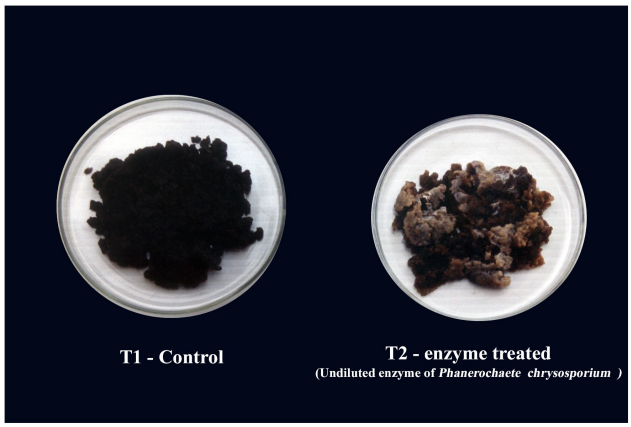
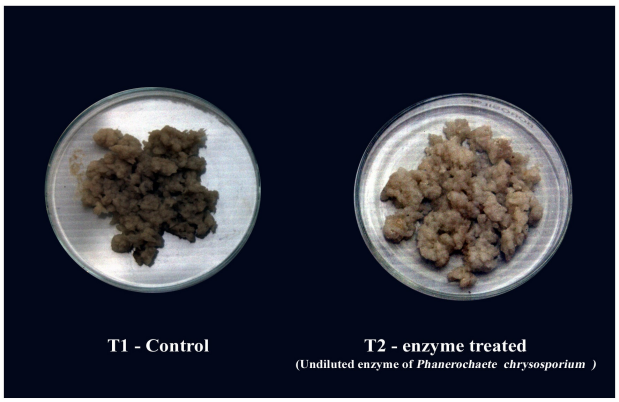


Fig 2 Bleaching of EP stage pulp using *Phanerochaete chrysosporium*



Conclusion

Pulp mill bleaching units are the most polluting sections in pulp and paper industries which have recently undergone enormous modifications to minimize their environmentally negative impacts (Okan *et al.* 2015, Ferre *et al.* 2011). The bleaching process stage of the pulp mill produces numerous organochlorine compounds that remain in the bleaching unit wastewater. According to Sharma *et al.* 2014 these organochlorine compounds cause genetic damages. The chlorine-free pulp bleaching applications has gained importance over the last decade in order to eliminate the deleterious effects of organochlorine compounds (Sharma *et al.* 2015). Thanks to their intensive lignin degrading potential with rich enzymatic actions, the white rot fungi have attracted extensive interest.

Thus, in this present study, using elite fungal enzymatic extracts for eco - friendly bio bleaching of kraft pulps had been undertaken, which would have industrial application.

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