

Production of Fungal Xylanase and Laccase Enzymes for Enzymatic Pre-bleaching Application

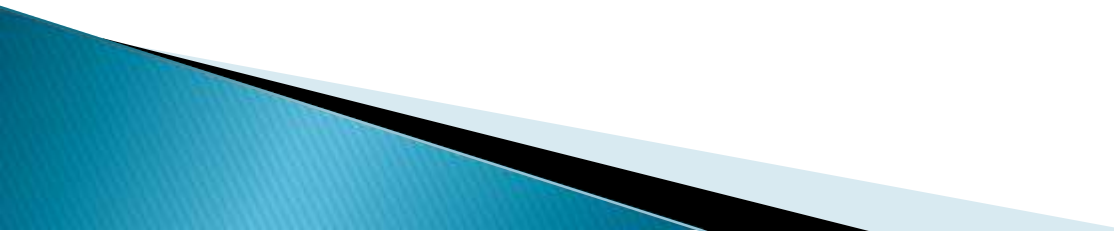
Rita Tandon, Anuradha V Janbade, Vipin Gupta

Presented by
Ms. Anuradha V Janbade
Scientist
Biotechnology Division

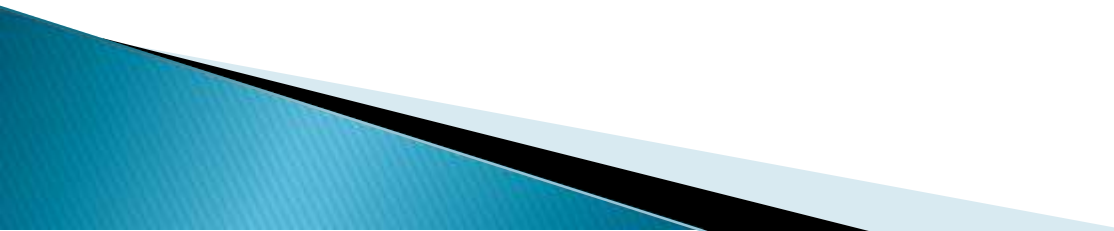


Central Pulp & Paper Research Institute
Saharanpur, (U.P.), India

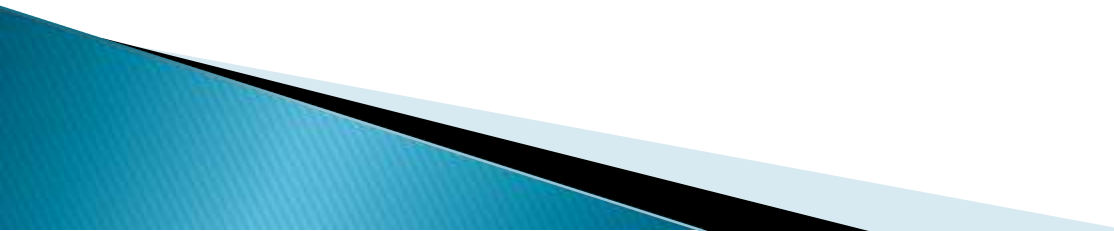
Background

- ❑ Pulp and paper mill industrial effluents contain toxic and harmful organic compounds as byproducts of pulping and bleaching processes.
 - ❑ Due to use of strong oxidants like Chlorine, chlorinated lignin and phenols are discharged into wastewaters.
 - ❑ To substitute chlorine and to implement environmentally sound bleaching sequences ,ECF & TCF bleach sequences are used.
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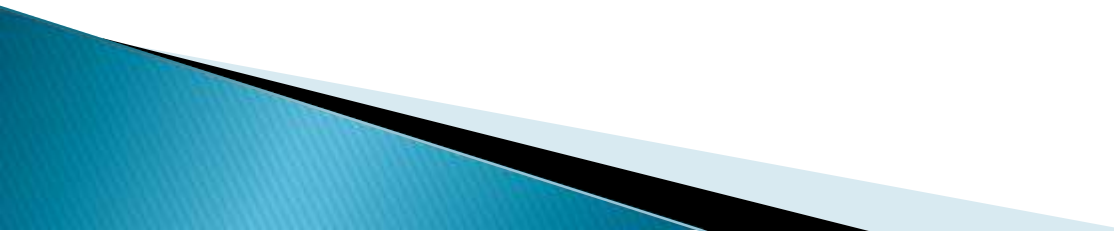
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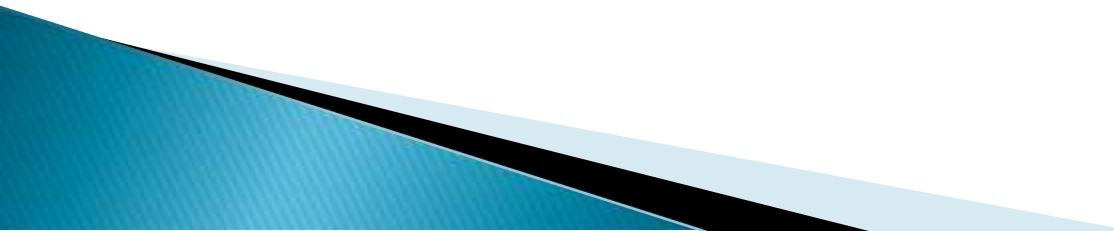
- ❑ Drawbacks of using these environmentally sound technologies was difficulty to attain high degree of brightness.
 - ❑ As Residual lignin and lignin-derived compounds are more recalcitrant to degradation in ECF & TCF bleaching.
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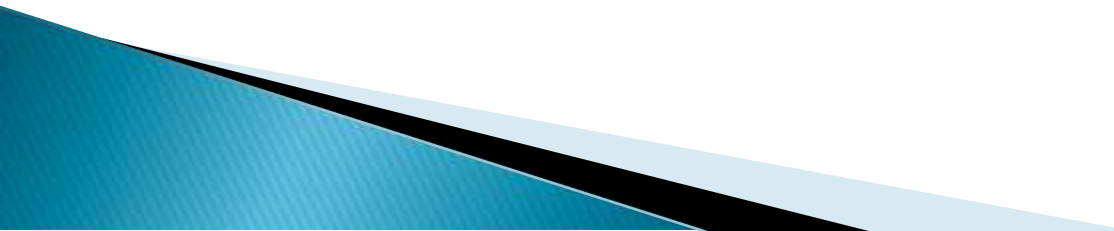
.....Background

- Enzymatic biobleaching is an efficient option to overcome these difficulties.
 - The need for adoption of greener technologies has led to a growing interest in the use of enzymes in the production of paper.
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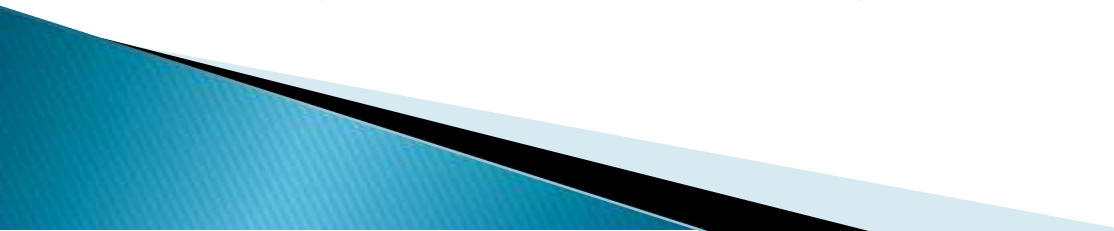
.....Background

- The application of enzymes like xylanases and laccases in pulp bleaching is important as they reduce release of pollutants during bleaching.
 - They also enhance the bleaching effect of chemical reagents by affording substantial savings.
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- Mixed enzyme systems consisting of a combination of hydrolytic and oxidative enzymes prove to be a promising strategy for achieving higher degree of pulp bleaching.
 - Xylanase exposes lignin which is degraded and removed in presence of laccases leading to improved level of delignification.
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- ❑ In the present study CPPRI made an effort to produce both xylanase and laccase enzymes from isolated fungal strain.
 - ❑ Produced enzymes were studied individually as well as in combination for their effect on improving the bleachability of the mixed hardwood pulp.
 - ❑ The effect of individual and mixed enzyme on the strength properties of pulp was also studied.
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Outline of Present Work

- Isolation of fungal strain for production of Xylanase and Laccase enzyme.
 - Production of Xylanase and Laccase enzymes indigenously.
 - Evaluation of the effect of Xylanase and Laccase enzyme application in bleaching of hard wood pulp individually and simultaneously in terms of brightness and strength properties.
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Production of Xylanase Enzyme

□ Isolation & Screening

- Fungus isolated from different sources like Garden soil, agriculture field soil and paper mill soil.
- 23 fungal colonies were isolated and screened by activity zone techniques with Congo red solution. 4 fungal colonies gave positive results for Xylanase

□ Production

Production was carried out in flask level with 100 ml malt extract broth inoculated with 10% of 5 day old fungal discs and incubated at optimum temperature (30°C) and optimum pH (6.0). Crude xylanase enzyme was extracted after 4 days

□ Enzyme Assay

The activity of crude Xylanase enzyme was achieved 150 U/ml.

Production of Laccase Enzyme

□ Isolation & Screening

- Fungus isolated from rotten wood by removing upper surface with sterilized forceps.
- The fungus was purified, grown and maintained on malt extract agar.
- Screening was done on MEA plates supplemented with Gallic acid, Phenol red and ABTS.

□ Production

- Production was carried out in mineral salt media (MSM) solution at pH 7.0 wheat bran used as carbon source.
- Temperature, pH and incubation time were optimized for better production of enzyme i.e. temperature 32°C, pH 5.0, days 3.

□ Enzyme Assay

The activity of crude laccase enzyme was achieved 1200 U/ml.

Xylanase and Laccase enzyme pre-treatment conditions for hardwood pulp for Bleaching

Particular	Control	Enzyme treated pulp			
		X.	L.	X. +L. (Full Dose)	X.+L. (Half Dose)
Enzyme dose (IU/gm)	-	15	25	15+25	7.5+12.5
Mediator (%)	-	-	0.2	0.2	0.1
Temperature (°C)	50	50	50	50	50
Treatment time (min)	180	180	180	180	180
Consistency (%)	10	10	10	10	10
Ph	7.4	7.6	7.3	7.2	7.8

Characterization of the unbleached pulp after enzymatic pre-treatment

Particular	Control	Enzyme treated pulp			
		X.	L.	X. +L. (Full Dose)	X.+L. (Half Dose)
Kappa no	14.00	13.32	12.92	12.60	12.75
Brightness %ISO	28.90	29.90	29.15	30.60	29.60
Yellowness % ISO	37.05	35.77	36.23	35.15	36.28
Whiteness % ISO	0.00	0.00	0.00	0.00	0.00

Characterization of enzyme treated and untreated bleached pulp

Particular	Control	Enzyme treated pulp			
		X.	L.	X. +L. (Full Dose)	X.+L. (Half Dose)
Brightness %ISO	83.05	84.45	84.65	85.35	84.90
Brightness improvement unit	-	1.40	1.60	2.30	1.85
Yellowness % ISO	7.01	6.90	6.25	5.30	5.70
Whiteness % ISO	71.20	71.85	72.40	75.80	75.20

Effect of enzyme treatment on Strength properties of the pulp

Particular	Control	Enzyme treated pulp			
		X.	L.	X. +L. (Full Dose)	X.+L. (Half Dose)
Burst index Pa.m ² /g	2.84	2.89	2.78	3.93	3.73
Tensile index, Nm/g	32.50	40.40	44.91	47.43	47.43
Tear index, Nm.m ² /g	7.19	6.99	6.21	8.08	6.96
Double Fold	21	12	23	22	22

Conclusion

- Indigenously produced xylanase and laccase enzyme by isolated fungal strain at optimized conditions showed good enzyme activity i.e. 150 IU/ml and 1200 IU/ml respectively.
- Both enzymes for pulp bleaching were found better when used simultaneously compared to individually in pulp bleaching.
- Brightness gain 2.30 unit when used simultaneously at 15+25 IU/gm(X+L) enzyme dose against control pulp.

.....Conclusion

- ❑ Brightness gain 1.85 unit when used simultaneously at 7.5+12.5 IU/gm (X+L) enzyme dose against control pulp.
- ❑ Brightness gain 1.40 and 1.60 unit when xylanase and laccase used individually at 15 and 25 IU/gm respectively dose against control pulp.
- ❑ Strength properties of the enzyme treated bleach pulp was improved when compared to untreated bleach pulp in respect to Burst index Tensile index Tear index, and Double Fold.

THANK YOU