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

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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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- 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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- 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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- 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.

- Mixed enzyme systems consisting of a combination of hydrolytic and oxidative enzyme 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.

- 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.

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.

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 achieved150 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 achieved1200 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.m2/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.m2/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.

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- 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