

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

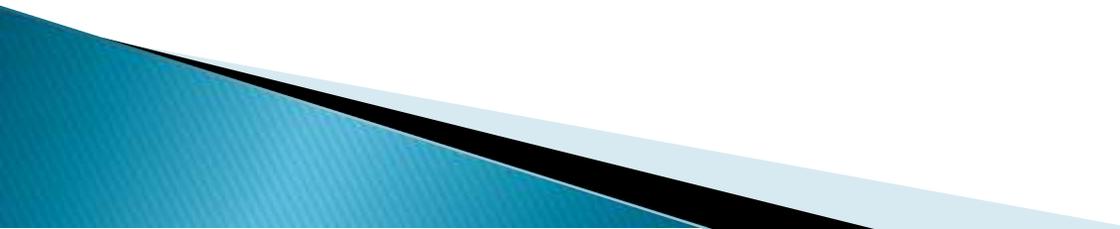
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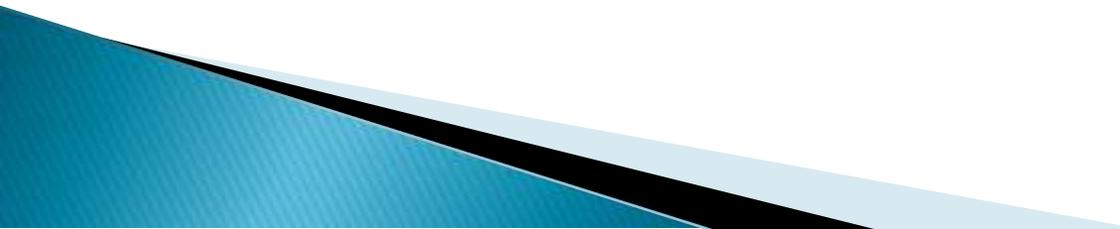


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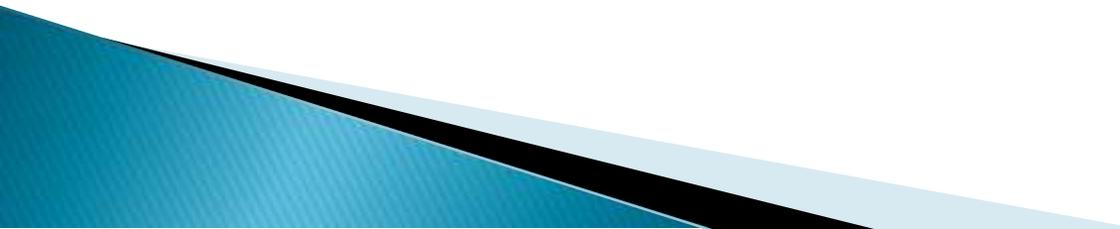
Background

- ❑ 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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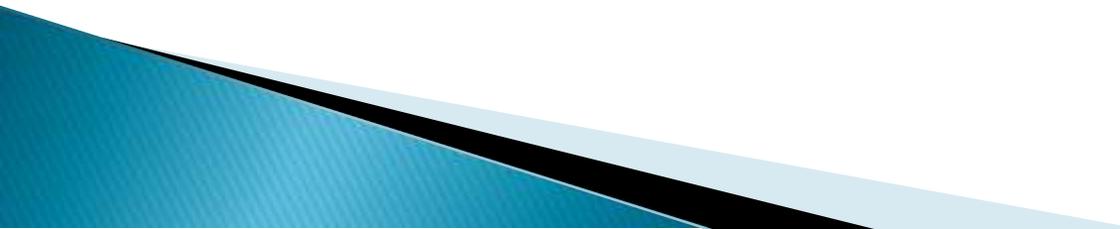
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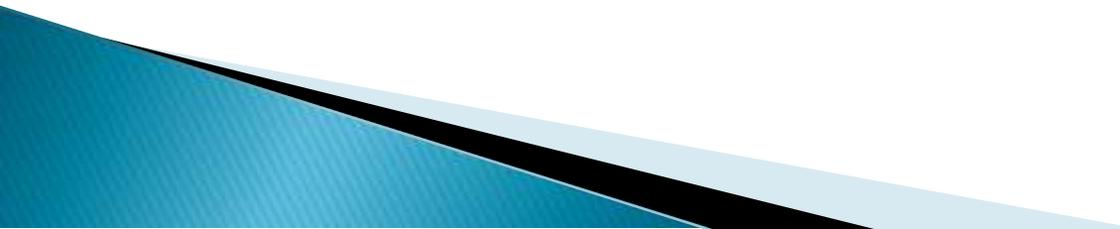
- ❑ Drawbacks of using these environmentally sound technologies was difficulty to attain high degree of brightness.
 - ❑ As Residual lignin and lignin-derived compounds are more recalcitrant to degradation in ECF & TCF bleaching.
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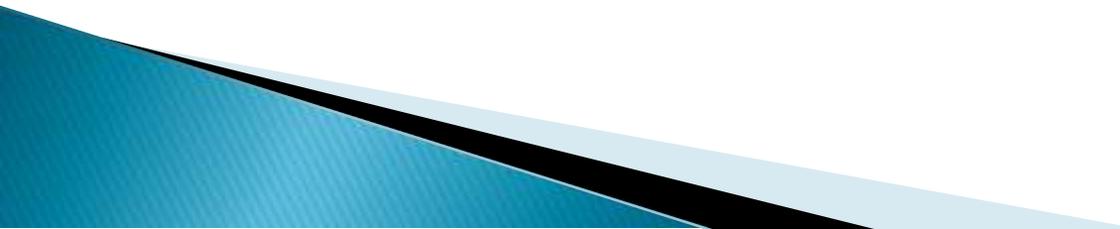
.....Background

- 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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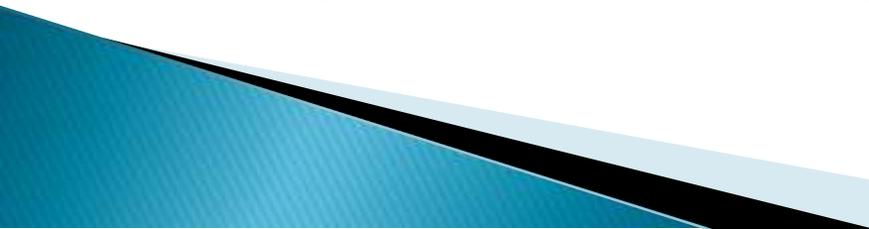
.....Background

- 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