CHAPTER 6

SUMMARY
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The major findings of this study entitled "Biobleaching of kraft pulp from some Indian trees and grasses and biocolour removal of effluents using white rot fungus *Phanerochaete chrysosporium* or other soil isolates", are summarised below.

6.1 Amongst 12 lignin degrading fungi i.e. eight soil isolates and four standard strains of *P. chrysosporium* (anamorph *S. pulverulentum*), screened for production of zones of bleaching on wood and grass kraft pulp sheets, only one isolate 'VJ₁' compared favourably with the four standard strains.

6.2 Amongst five strains obtained after screening, two organisms i.e. *P. chrysosporium* K-3 and soil isolate VJ₁ were selected based on cellulolytic and ligninolytic enzyme profiles and biobleaching characteristics of wood and grass pulps under three cultural conditions viz. surface, suspended and shake. Growth with *P. chrysosporium* K-3 in surface culture in case of wood pulp resulted in 53% kappa number reduction (measure of delignification) and 37.3% viscosity loss whereas isolate VJ₁ caused 43.4% reduction in kappa number accompanied by 22% viscosity loss in case of grass pulp in 3 days.

Out of the three cultural conditions, surface culture invariably gave the best results in terms of highest
delignification of both wood and grass pulps by all five strains.

6.3 Screening of kraft pulps made from eight different raw materials for their biobleaching by \textit{P.chrysosporium} K-3 showed higher kappa number fall in 3 days in case of bamboo, eucalyptus and khar grass pulps by 66.8\%, 54.8\% and 54.0\% respectively. Decreased viscosity and increased copper number were common feature accompanying the fall in kappa number in all cases.

6.4 Effect of carbon source, buffering agent, nitrogen source, surfactant, chelators and glycols was studied to optimise the nutritional medium for biobleaching of eucalyptus and khar pulps by \textit{P. chrysosporium} K-3. Maximum kappa number fall was observed with 1\% glucose, 10mM dimethyl succinate (pH 4.6), ammonium tartarate (2.4 mM N) and 0.1\% tween 80. Addition of chelators and glycols did not show any positive effect on bleaching.

Biobleaching of eucalyptus kraft pulp by \textit{P.chrysosporium} K-3 under optimised nutritional conditions resulted in 55\% reduction in kappa number alongwith a marked increase in brightness (76\%) in 3 days. Further detailed studies were carried out with eucalyptus pulp only.

6.5 The study of handsheet properties of biobleached eucalyptus pulp showed that burst factor, tensile strength, breaking length and gurley porosity of pulp were improved following fungal treatment. Energy requirements for refining the biobleached pulp as measured by number of revolutions in PFI mill to develop a given freeness were
decreased by 21.7% compared to that of control.

Refining of both unbleached and biobleached pulp resulted in an improvement in handsheet properties but the rate of improvement was much slower in biobleached pulp. Thereby biobleached refined pulp had poorer strength properties than unbleached refined pulp.

6.6 Treatment of eucalyptus pulp for different incubation intervals showed that biobleached pulps obtained after 3 day incubation with *P. chrysosporium* had lowest kappa number, highest brightness and maximum level of most of strength properties both with and without refining.

6.7 Highest improvement in handsheet properties of biobleached pulp took place when the pulp was refined to 400 ml freeness. On subsequent refining to 350 ml and 300 ml, biobleached pulp did not show any marked increase in properties except gurley porosity.

6.8 Biobleaching of eucalyptus pulps inoculated with different mycelial concentrations showed higher kappa number reduction and brightness improvement at 5% concentration as compared to that at 2.5% after three days incubation. Increase in inoculum concentration to 7.5% and 10.0% did not give further improvement in delignification whereas minor changes were observed in case of handsheet properties with and without refining.

6.9 Reduction of glucose concentration from 1.0% to 0.5% led to 32.8% lesser reduction in kappa number and 50% lesser improvement in brightness. On the other hand increasing the glucose concentration from 1% to 2% and 4% did not result in
any improvement in both the parameters. However, significant improvements in some of the handsheet properties like burst factor, tear factor, zero span tensile strength, fibre strength factor and fold were observed at 2% glucose level.

6.10 Lowering of initial kappa number of eucalyptus pulp from 26.5 to 18.1 resulted in faster biobleaching process as indicated by saving of one day incubation period (from 3 to 2 days) to achieve same increase in brightness (20 units). The trend of changes in handsheet properties on biobleaching and refining was almost similar in pulps of different initial kappa numbers.

6.11 Prerefining of pulp prior to biobleaching showed that the prerefined pulp upto 500 ml freeness helped in improvement of bulk, burst factor, tear factor and breaking length with additional refining at 400 ml freeness.

6.12 Most optimum conditions for biobleaching of eucalyptus pulp of initial kappa number 26 to 29 were, prerefining of the pulp to 500 ml freeness, inoculation with 5% fungal mycelium, incubation for 3 days in the presence of 1% glucose and final refining to 400 ml freeness for handsheet preparation.

6.13 Alkaline extraction of the biobleached pulp with 1% sodium hydroxide resulted in 8 to 10% higher values of burst factor, tear factor, breaking length and tensile strength and 100% increase in fold in refined state. The pulp obtained under these conditions showed 4 units increase in brightness and 12.8% fall in kappa number.
6.14 Single stage chemical bleaching of biobleached pulp with three different agents i.e. calcium hypochlorite, chlorine dioxide and hydrogen peroxide showed highest fall in kappa number (48.6%) and increase in brightness (72%) after treatment with hydrogen peroxide. Peroxide bleaching also resulted in 11.8%, 7.0%, 11.6% and 12.1% improvement in burst factor, tear factor, tensile strength and breaking length of biobleached pulp in refined state.

6.15 Multistage chemical bleaching of biobleached pulp and chlorine bleached pulp in two sequences i.e. HH (Hypochlorite, Hypochlorite) and DED (Chlorine dioxide, Alkaline extraction and Chlorine dioxide) showed better response of both pulps to HH bleaching with final brightness of both the pulps being same i.e. 75 units with almost similar changes in strength properties.

6.16 Analysis of handsheets prepared after three stage biobleaching in BEP sequence (Biobleaching, alkali extraction, peroxide bleaching) with biobleaching process carried out under most optimum conditions showed brightness level of 74% photovolt and good strength properties both in unrefined and refined states compared to that of control unbleached pulp.

6.17 Maximum stability of crude ligninase preparation of *P. chrysosporium* K-3 for 1 hour was observed at pH 3.5 and at 25°C.

Reaction of crude ligninase with unbleached eucalyptus pulp resulted in release of some compounds which caused changes in optical densities and spectral patterns.
Alkaline extraction fluid of the enzyme treated pulps showed an increase in ratio of optical densities at 280 and 260 nm as well as \( \lambda_{\text{max}} \) of the UV scan. However, optimised enzyme treatment could not cause any changes in kappa number and brightness of the pulp.

6.18 P. chrysosporium K-3 caused the most rapid decolourisation of alkaline extraction stage effluent out of four standard strains and an isolate. It could remove 75% colour in 7 days at 39\(^\circ\)C under stationary culture.

6.19 Presence of an additional carbon source was found to be essential for decolourisation by P. chrysosporium under stationary culture. Highest colour reduction of 74% took place at 1% glucose concentration as decolourisation efficiency had a direct relationship with glucose concentration. Replacement of glucose with pith, cellulose and primary sludge showed primary sludge to be most effective giving 80% decolourisation in 7 days.

6.20 Higher the initial colour concentration of the effluent, more the average number of colour units removed per day during 7 day treatment, the maximum and minimum values being 422 and 94 colour units in case of effluents with initial colour of 6000 and 1000 units. However, the percent colour removal was highest i.e. 80.4% in effluent of 4000 initial colour units.

6.21 Maximum colour reduction of Alkaline extraction stage (AES) effluent took place at 39\(^\circ\)C with 75% colour removal by P. chrysosporium K-3. Colour removal was 59.5% at 32\(^\circ\)C and 53.0% at 25\(^\circ\)C.
The scale up of biocolour removal experiment in a 6.0 litre stirred LKB fermenter showed that about 80% colour could be removed after 96 hours of treatment with *P. chrysosporium* K-3 in presence of 1% glucose under submerged fermentation.