MAJOR OUTCOMES OF THE STUDY

- Screening of white-rot fungi for potential bio pulping is important to select efficient lignin degraders which are capable of accomplishing delignification in a reasonable period of time and which are also capable of performing selective delignification which leaves the cellulose fibers intact. The results of the present qualitative analysis indicated that among the three species of *Trichoderma*, *T. viridae* and *T. harzianum* produces all the three enzymes (ligninolytic, cellulolytic and xylanolytic enzymes) where as *T. reesei* produces only xylanolytic enzymes. All the white rot fungi produced cellulase free ligninolytic enzymes. Xylanolytic enzymes were found to be absent in *Pleurotus florida* and *Pleurotus ostreatus*.
- The paired interaction test between 12 fungal isolates revealed five different types of interactions *viz*. mutual intermingling, partial mutual intermingling, Invasion / replacement, deadlock at touching point and dead lock at a distance amongst which mutual intermingling and partial mutual intermingling could be considered as the best compatible coculture. From these potential compatible cocultures IL+DC, IL+PHE, IL+PS, PS+DC and PS+PHE were identified which used for the further quantitative analysis.
- Result of quantitative analysis indicated that monoculture of *I. lacteus* and *P. sajorkaju* showed maximum enzyme activity in 30 days of incubation period and in *D. confragosa* and *P. pectinatus* maximum enzyme activity was noticed on 25th days. Compared to monoculture, coculture/Mixed cultures produced enzymes which were significantly higher than monocultures within a shorter incubation period of 20 days. All the monocultures and cocultures of fungal isolates producing cellulase free major lignocellulolytic enzymes proved to be beneficial in bio pulping and bleaching for paper and pulp industry.

- All the enzymes activities were assessed at different temperatures and pH to identify the optimum temperature and pH at which enzyme activity was maximum. The maximum enzyme activities were observed at 25˚C temperature and pH 5. However enzymes secreted by the fungal isolates were found to be stable up to 45˚C. At high acidic pH enzyme activities were noticed to have reduced but the activities were stable up to pH 7.
- The degradation pattern of both the fungi on *Eucalyptus globulus* was studied. Both the fungi degrade lignin selectively in the initial stages as middle lamella were found to be dissolved and separation of fiber cells were noticed. However degradation pattern of both fungi were different as *I. lacteus* caused degradation of parenchyma cells prominently and loosening of fiber cells due to degradation of middle lamella, a typical feature characterizing it to be a selective white rot fungus whereas *D. confragosa* initiates formation of bore holes at advanced stages of decay showing dual pattern of decay ie, selective in the initial stages and simultaneous at later stages. Results of biochemical analysis very clearly depicted that degradation of wood components was significantly higher in the coculture.
- Amongst the monocultures studied maximum enzyme activity of all the enzymes were found in *I. lacteus* and *D. confragosa*. Coculture of these provided the best enzyme activities amongst all the experimented samples and hence monocultures and coculture of *I. lacteus* and *D. confragosa* were selected for the further experiments.
- Enhancement of enzyme activity by supplementing different chemical enhancers with varying concentrations indicated 1% and 4% ethanol, 12mM and 16mM veratryl alcohol, 30µM xylidine, 0.6g/L yeast extract and 0.4g/L peptone to be the best enhancers of all the enzyme activities.
- Enzyme enhancement experiments by supplementing different lignocellulosic materials in various concentrations were studied. Results indicated 5% apple peels, 4% banana

peels, 6% mandarin peels and 5% ash gourd pulp to enhance all the lignocellulolytic enzyme activities to the maximum.

- Further bio pulping experiments with *Eucalyptus globulus* wood blocks and pulp were carried out supplementing it with the best concentrations of chemical enhancers. Results indicated 16mM veratryl alcohol and 30µM xylidine to be the best chemical inducers and 5% ash gourd pulp as the best lignocellulosic material which enhances the enzyme activities.
- Introducing biopulping process (fungal pretreatment) prior to chemical or mechanical pulping will lead to separation of cellulose fibers due to effective loss of lignin and hemicellulose, so requirement of chemicals and electrical energy in the successive pulping process will reduced which ultimately would result in lesser pollution of the environment.
- The results obtained from the present study have a very high potential of practical application value as well of academic importance. Enzymes produced by the fungi will help in the process of bio pulping in which lignin is degraded and cellulose fibers can be easily separated, where as xylanase enzyme plays an important role in the bio bleaching process in which the separated fibers would be brightened and not yellow.
- Ligninolytic and xylanolytic enzymes and enhancement of the enzymes can be utilized in other biotechnological applications like biodecolorization (Dias *et al* 2013), bioremediation (Beltz *et al* 2001, Cheong *et al* 2006), Bio-fueling, effluent treatment and agro-waste treatment and industries like textile and food industries (Subramaniyn and Prema 2002).
- Similiarly in food industries especially xylanase enzymes are used for clearing juices and wine (Wong *et al* 1988), for extracting coffee, plant oils and starch (Biely 1991), for improving nutritional qualities of grain feed (Malathi and Devegowada 2001) and it also

increases volume of baked bread if used for baking process (Harbak and Thygesen 2002).

- The potential xylanase producers identified in the present study (*Irpex lacteus* and *Daedeleopsis confragosa*) and conditions identified to enhance its production will enable industrialists to apply this biotechnological technique in obtaining an increased production of the enzymes at a cheaper rate.