CONCLUSION
In recent years, research on lignin degrading enzymes has been greatly intensified because of their potential applications in a variety of biotechnological processes. These applications include biotransformation of lignocellulosic biomass to feeds, fuels and chemicals, biopulping, biobleaching of paper pulps, decolorizing and detoxifying kraft bleach effluents and degradation of highly toxic chemicals such as dioxins, polychlorinated biphenyls, various dye pollutants and polyaromatic hydrocarbons released in the environment. Lignolytic fungi as such can also be of great help in bioremediation processes like removal of toxic metals from the environment.

Basidiomycetous fungi involved in white rot decay of wood are known to play a major role in the mineralization of the lignin polymer to carbon dioxide and water in the terrestrial environment, while bacteria are believed to be relatively unimportant in this process. Basidiomycetous fungi are also capable of mineralizing a wide variety of toxic xenobiotics because the enzymes produced are non-specific and extracellular. Three major classes of enzymes designated as laccases, manganese peroxidases (MnPs) and lignin peroxidases (LiPs) are important in the degradation of lignin and xenobiotics. Laccases are copper containing oxidases while LiPs and MnPs are heme-based peroxidases. Some wood degrading fungi
contain all three classes of lignin modifying enzymes, while the others contain one or two of these enzymes.

In the present study, screening was carried out to obtain fungal isolates from rotting wood, plant litter and soil samples from paper mill premises. Among eighteen isolates, white rot fungus BDT-14 was selected for further study on the basis of a positive Bavendum reaction. The isolate BDT-14 is confirmed as a Basidiomycete, having arthrospores and clamp connections. The isolate was maintained on Sabouraud’s Dextrose Agar medium and subcultured every month.

BDT-14 was checked for the production of lignolytic enzymes viz. laccase, lignin peroxidase and manganese peroxidase. Amount of laccase produced was greater than the amount of lignin peroxidase during growth of fungus in Asther’s medium. Manganese peroxidase was not detected in the medium.

Optimization of various parameters was carried out. Increase in amount of carbon source, showed increase in enzyme production. Excess carbon source had no inhibitory effect on enzyme production. As natural habitat of the fungus was wood, good growth and enzyme production was confirmed under nitrogen limiting condition. Effect of surfactants and
different phosphate sources was checked for the production of enzymes. Addition of surfactants to the growth medium resulted in a marginal enhancement of the enzyme activities. Amongst the surfactants tested Tween-80 gave maximum enhancement.

Variety of agro-wastes was utilized for the growth of the fungus and thereby getting good amount of enzymes at very low cost. Laccase and lignin peroxidase production was compared during growth on neem hulls, bagasse, coconut coir and banana pseudostem as solid state fermentation. Even though neem is considered to have many antimicrobial agents, maximum growth and enzyme production was found on neem hulls. Bagasse was also giving good growth but enzyme production was comparatively less.

Precipitation of extracellular culture filtrate with acetone and ammonium sulfate resulted in to partial purification of enzymes. These enzymes were further purified by passing through Sephadex G-75 column and molecular weight determination was done by PAGE. Interestingly the protein fraction obtained from the column when stained for activities of laccase and lignin peroxidase after electrophoresis on native-PAGE, revealed single band with apparent molecular weight of 70 KD. This suggested possibility of having a protein fragment with both the activities or two
enzyme proteins having same molecular weight which is so far not reported especially with laccase and LiP from any other organism.

Since BDT-14 showed the ability to degrade lignin, it was tried for the PAHs degradation. Naphthalene, phenanthrene and anthracene mineralization was observed with BDT-14. The fungus was able to grow with very low concentration of carbon source and degrade these PAHs. Metabolite generation and thereby PAH degradation was confirmed performing thin layer chromatography and HPLC.

White rot fungi are known to completely decolorize and degrade various dyes. Present study has investigated decolorization of eight industrial dyes viz. Acid Red, Acid Brown, Direct Blue, Reactive Black, Reactive Golden Yellow HR, Reactive Red, Reactive Violet and Reactive Orange. All the eight dyes showed decolorization in liquid medium. Maximum decolorization was observed for Acid Red, Acid Brown and Direct Blue. Decolorization was observed on agar plates also. During growth on dyes it was found that fungus is capable of using dye as the sole source of carbon and produced both the enzymes.

Toxicity of the treated and untreated samples was compared using *Daphnia* as test organism to show the efficacy of the treatment with
lignolytic enzymes. Toxicity of the sample after enzymatic treatment was greatly reduced compared to untreated sample.

Decolorization was confirmed with partially purified enzymes. 70% decolorization was observed with Acid Brown and Direct Blue whereas 100% decolorization was observed with Acid Red within 24 hours. Partially purified enzymes were also tried on solid medium with Acid Red, which showed clear colorless zone surrounding the enzyme containing wells.

Looking to the soil-colonizing tendency of the fungus, BDT-14 grown on neem hulls was used for bioremediation of soil, artificially contaminated with Acid Red dye. After 42 days of incubation, bioremediation resulted in to a complete removal of the dye from soil samples. Similarly, when coconut coir was utilized as support material and to provide nutrition, for the growth of fungus and bioremoval of dye, 100% decolorization was achieved after 21 days.

A laboratory scale bioreactor was used to assess decolorization by the fungus immobilized on polyurethane foam. Under this condition, complete decolorization of artificial effluent with 100 ppm Acid Red concentration was successfully achieved within 48 hours.
Because of the characteristic feature of white rot fungus to selectively degrade lignin, it has been exploited in biobleaching of kraft pulp, which has advantages over the chemical bleaching. 67.3% decrease in kappa number of wood pulp was recorded as a result of fungal growth and action of lignolytic enzymes.

In addition to extracellular enzymes, white rot fungus itself has significance in maintenance of pollution free, clean environment. Due to their cell wall structure, fungi exhibit high metal binding capacity and are therefore useful in removing heavy metals from the heavy metal containing waste, thus decreasing the toxicity of effluent as well as recovering some costly metals back. Cr (VI) adsorption study on both live and dead biomass was carried out in detail. When 10% dead cell mass was used, 100% Cr removal from 100 ppm potassium dichromate solution and 49% removal from 500 ppm potassium dichromate solution was observed within 24 hours in shaking condition. When fungus was grown in presence of heavy metals, it was observed that Cr (VI) had highest toxic effect with 100 ppm MIC. Among the other metals studied for their effect on growth of the fungus, Zn, Cu, Ni and Co showed toxic effect in decreasing order where as Pb had stimulatory effect on growth of the fungus.
The main objective of the study to screen out fungal strain preferably white rot fungus that would make good candidate for bio-remediation of xenobiotics, employing extracellular lignolytic enzymes has been fulfilled. Bioremediation by white rot fungi has opened up new opportunities to overcome the limitations of conventional methodologies for the treatment of xenobiotics and thereby cleaning up the environment, maintaining it eco-friendly.