Chapter 7.

SUMMARY AND CONCLUSION
The thesis includes the experimental methodology and results of optimization and characterization of laccase from an actinomycete strain Streptomyces psammoticus. The application of enzyme in pollution abatement was also evaluated with special reference to the degradation of dyes and phenols.

7.1. SUMMARY

- Twenty actinomycete cultures were isolated from the marine and mangrove regions along the west coast of India.
- Six isolates showed positive result in the primary screening and selected for secondary screening by SmF.
- Six isolates produced LiP; four isolates produced laccase and one isolate (NJP 49) produced LiP, MnP and laccase in SmF medium.
- The isolate NJP 49, identified as Streptomyces psammoticus was selected as the best strain, based on the enzyme yield.
- Laccase was selected for further work in view of its role in industrial applications.
- Coffee pulp was identified as the best substrate for laccase production in SmF.
- The optimization studies revealed that the laccase production was maximum at pH 7.5 and temperature 32 °C.
- Salinity of the medium was also observed to be influencing the enzyme production. Maximum production was observed in media with 50 % seawater.
- An agitation rate of 175 rpm and 15 % inoculum were the other optimized conditions for maximum laccase yield.
- Pyrogallol and para anisidine proved to be the best inducers for laccase production by this strain.
- Statistical optimization was also done in SmF. Initial screening of production parameters was performed using a Plackett - Burman design.
The variables with statistically significant effects on laccase production were identified and optimized further by Box-Behnken design.

The statistical optimization by response surface methodology resulted in a three-fold increase in the production of laccase.

Optimization of laccase production in SSF was carried out by conventional as well as statistical approaches.

Rice straw was identified as a suitable substrate for laccase production in SSF, followed by coffee pulp.

Other optimized conditions under SSF were particle size - 500 – 1000 μm; initial moisture content - 65 %; pH of moistening solution - 8.0; incubation temperature - 32 °C and inoculum size – 1.5 x 10^7 CFU.

Yeast extract served as the best nitrogen source. No enhancement in enzyme yield was observed with carbon supplementation.

The level of yeast extract, inoculum size and copper sulphate were optimized statistically using central composite design.

Statistical optimization resulted in three-fold increase in laccase activity as compared to the unoptimized medium.

Enhanced production of laccases from *S. psammoticus* in SSF was carried out using two different strategies; laccase inducers and scale-up process.

Laccase yield was enhanced by a wide range of aromatic inducers. The best inducer was pyrogallol.

Scale-up studies in packed bed bioreactor was performed at different aeration rates. 1.5 vvm aeration was identified as the optimum condition for laccase production in column bioreactor.

Fermentation was carried out in bioreactors in the presence of 1 mM pyrogallol, which resulted in 3.9 fold increase in laccase yield.

Laccase from *S. psammoticus* has been purified to homogeneity through anion exchange and gel filtration chromatography steps with an overall purification fold of 12.1.

The molecular mass of the purified laccase was about 43 kDa. The pI was 7.9.
- The enzyme was active in the alkaline pH range with pH optima at 8.5 and 97% activity retention at pH 9.0. The enzyme was tolerant to NaCl concentrations up to 1.2 M.
- The optimum temperature was 45 °C. The enzyme was stable in the pH range 6.5-9.5 and up to 50 °C for 90 min.
- Purified laccase was inhibited by all the putative laccase inhibitors while the enzyme was activated by metal ions like Fe, Zn, Cu, Na and Mg.
- The enzyme showed lowest $K_m$ value with pyrogallol (0.25 mM) followed by ABTS (0.39 mM).
- The purified enzyme was a typical blue laccase with an absorption peak at 600 nm.
- The enzyme was a glycoprotein with a carbohydrate content of 10%. The enzyme contained 3.2 copper ions per molecule.
- The dye decolourization ability of S. psammoticus was evaluated using ten different dyes. RBBR was effectively degraded by S. psammoticus.
- S. psammoticus immobilized on PUF removed 89.2% of total phenolics and 77.2% COD from the synthetic phenol solution.
- Laccase immobilized on copper alginate beads removed 72% of the colour and 69.9% of total phenolics after initial run of 6 h.
- Reusability of the immobilized matrix was studied for up to 8 successive runs, each run with duration of 6 h.
- The degradation of phenolic compounds by immobilized laccase was evaluated and confirmed by thin layer chromatography and nuclear magnetic resonance spectroscopy.
- Four laccase mediators such as ABTS, HOBT, aniline and pHBA were used for mediator based decolourization of azo dyes.
- HOBT was identified as the best mediator for laccase from this strain. Acid orange, Methyl orange and Bismarck brown were decolourized at the rates of 86, 71 and 75% respectively by HOBT.
7.2. CONCLUSION

The present study has identified an actinomycete culture (*S. psammoticus*) which was capable of producing all the three major ligninolytic enzymes. The study revealed that least explored mangrove regions are potential sources for the isolation of actinomycetes with novel characteristics. The laccase production by the strain in SmF and SSF was found to be much higher than the reported values. The growth of the organism was favoured by alkaline pH and salinity of the medium. The enzyme also exhibited novel characteristics such as activity and stability at alkaline pH and salt tolerance. These two characters are quite significant from the industrial point of view making the enzyme an ideal candidate for industrial applications. Many of the application studies to date are focused on enzymes from fungal sources. However, the fungal laccases, which are mostly acidic in nature, could not be used universally for all application purposes especially, for the treatment of effluents from different industries, largely due to the alkaline nature of the effluents. Under such situations the enzymes from organisms like *S. psammoticus* with wide pH range could play a better role than the fungal counterparts. In the present study, the ability of the isolated strain and laccase in the degradation of dyes and phenolic compounds was successfully proved. The reusability of the immobilized enzyme system made the entire treatment process inexpensive. Thus it can be concluded from the present study that the laccase from this organism could be hopefully employed for the eco-friendly treatment of dye or phenol containing industrial effluents from various sources.