CHAPTER 5
SUMMARY AND CONCLUSION

Lignin is the second most abundant biopolymer in nature after cellulose. Lignin is a complex three dimensional nonstereoregular polymer composed of phenyl propanoid units linked through several major types of carbon-carbon and ether bonds. It is structurally evolved and designed to protect from microbial attack and is recalcitrant in nature. The biodegradation of lignin involves armoury of oxidative and extracellular enzymes secreted by lignolytic organisms. Lignolytic enzymes have great potential for industrial applications and include mainly Laccases (Lac), Lignin peroxidases (LiP) and Manganese peroxidases (MnP).

Laccase (Benzenediol:oxygen oxidoreductases; EC 1.10.3.2) is a multi copper blue phenol oxidases capable of oxidizing ortho and para-diphenols and aromatic amines by removing an electron and proton from a hydroxyl group to form a free radical.

Lignin peroxidase (Diarylpropane:oxygen,hydrogen-peroxide; oxidoreductase EC 1.11.1.14) is a glycoprotein with heme group in its active center capable of cleaving aromatic rings (Via) one electron subtracation and subsequent incorporation of oxygen. It also oxidizes phenolic and non phenolic compounds, amines, aromatic ethers to form a free radical.

Manganese peroxidase (Mn (II): hydrogen-peroxide oxidoreductase EC 1.11.1.3) is a heme containing peroxidase that oxidizes phenolic Mn$^{2+}$ to Mn$^{3+}$, using H$_2$O$_2$. 

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as an oxidant. Activity of the enzyme is stimulated by simple organic acids which stabilize the Mn$^{3+}$.

These enzymes lack substrate specificity and are thus capable of degrading a wide range of xenobiotics. However, distribution profile of lignolytic enzymes and metabolic pathways of lignin biodegradation vary from one organism to another. Knowledge on these aspects will be useful to application of the enzymes. In nature both cellulose and lignin co-exists together in litter. Due to encounter of these two components in nature the fungal cultures are tested for their cellulolytic activities.

In the light of importance of lignolytic enzymes in bioremediation and in industry the present study is focused on the production of lignolytic enzymes by Stereum ostrea in comparison to the reference culture Phanerochaete chrysosporium in submerged fermentation and the ability to decolourize textile dye by Stereum ostrea.

Laccase production by Stereum ostrea and Phanerochaete chrysosporium were visualized on agar plates containing guaiacol as a substrate because of formation of reddish brown zones in the medium due to oxidative polymerization of guaiacol. As it was not clear about production of laccases in quantitative terms from plate experiments. Studies were further carriedout with these cultures in submerged fermentation in flasks for laccase production.

Laccase production was observed by both Stereum ostrea and Phanerochaete chrysosporium on 2nd day and it reached maximum on 4th day of incubation in
culture broth and then declined. Peak laccase yields by *Stereum ostrea* on 4\(^{th}\) day of incubation at pH 6.0 and temperature of 30°C were higher in comparison to the reference culture *Phanerochaete chrysosporium*. It shows that *Stereum ostrea* is a better producer of laccase than the reference culture.

Activities of lignin peroxidase and manganese peroxidase were not detected in the culture filtrates of *Stereum ostrea*, but the reference culture *Phanerochaete chrysosporium* displayed maximum activities of lignin peroxidase and manganese peroxidase on 4\(^{th}\) day of incubation and enzyme activity gradually decreased.

Maximum filter paperase, carboxy methyl cellulase and β-D-glucosidase activities were obtained on the 6\(^{th}\) day of incubation in Koroljova-Skorobogat'ko *et al.*, medium at pH 6.0 and temperature of 30°C in *Stereum ostrea* culture than the reference culture *Phanerochaete chrysosporium*. There was gradual drop in enzyme activity until the 10\(^{th}\) day of incubation in both the cultures.

The presence of lignolytic system in *Stereum ostrea* was confirmed by decolourisation of Remazol orange-16 dye on solid medium. Similar results of decolorization of dye were reproduced when both cultures were grown in liquid medium amended with dye at different concentrations. Decolorization activity was observed for 10 days and the decolorization activity was expressed in terms of percentage. *Stereum ostrea* showed significant decolorization towards Remazol orange-16 dye.
Conclusions

The following are the conclusions of the present study:

- *Stereum ostrea* has proven to be better laccase producer than the reference culture *Phanerochaete chrysosporium*.
- LiP and MnP were not detected in culture filtrates of the *S. ostrea* under conditions used in the present but these enzyme activities were displayed by the reference culture *P. chrysosporium*.
- Laccase appeared to be mainly dominant in lignolytic system of *S. ostrea*.
- Both cultures displayed activities of filter papaerase, β-endoglucanase and β-glucosidase indicating that they are also cellulolytic in nature.
- Based all the tested parameters, *Stereum ostrea* was shown to be lignocellulolytic in nature.
- Presence of lignolytic system in *Stereum ostrea* was further confirmed by Remazol orange-16 dye and maximum decolorization was observed with *Stereum ostrea* than with the reference culture *Phanerochaete chrysosporium*.