
Summary and Conclusion

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Laccases catalyze the oxidation of a wide range of substrates preferentially phenolic compounds. This property of laccase is being exploited in various biotechnological processes. Hence the study on laccase producing organisms is intensified in recent years. The optimization conditions for microbial growth and maximum laccase production is essential for the use of enzymes in various fields.

There are only few reports on the optimization of laccase production from the environmental samples. Hence in the present study the bacterial species isolated from the textile effluent samples were selected and subjected to different environmental conditions using one time one factor approach to achieve maximum laccase production.

The selected physicochemical conditions were further optimized using the most powerful statistical tool Plackett-Burman Design. The physico chemical conditions selected for the study were namely inoculum age, temperature, carbon source, nitrogen source, inducers, metal ions and surfactants.

One time one factor approach was carried out to optimize the physico chemical conditions by varying the level of one factor and keeping the other factors at a certain constant level. The effect was observed in terms of bacterial growth and laccase production.

The Plackett-Burman Design is a type of two level screening design which allow the study of 'n' number of factors with ' $n+1$ ' + '2' trials. In the

present study 11 components were used to develop the statistical design. They were glucose, pH, incubation period, inoculum size, magnesium sulphate, trace element solution, phenol, agitation, yeast extract, sodium acetate and ammonium sulphate.

Low and high settings of each of these components were used to prepare a combination of twelve trials of production medium. Biomass and Laccase activity were determined for each trial.

The results obtained are summarized as follows:

One time one factor approach

- The bacterial growth was found to be significantly increased with increasing inoculum age from 6hrs to 24hrs.
- An increase in temperature from 27⁰ C to 37⁰ C significantly increased the growth and Laccase activity and further increase in temperature from 37⁰ C to 47⁰ C significantly reduced both growth and Laccase activity.
- Both inorganic and organic carbon sources used in the present study supported growth of the bacteria.
- Glucose was found to be the best organic carbon source for laccase production by the isolated bacterial strain when compared to the other organic carbon sources.
- Inorganic carbon source namely sodium acetate in the culture medium significantly increased laccase activity when compared to sodium citrate. The Laccase activity was found to be significantly increased with the sodium acetate as carbon source in the medium than with glucose as carbon source in the medium.

- All the selected nitrogen sources showed no inhibitory effect on the growth of bacteria. The maximum growth of bacteria was supported by potassium nitrate. Ammonium sulphate was found to be less favorable for bacterial growth.
- All the selected inducers in the medium significantly decreased the growth of bacteria except RBBR.
- Among the twelve selected inducers only phenol was found to significantly increase laccase activity when compared to other inducers.
- The bacterial growth was not altered when the inoculum size was increased from 0.1ml to 1ml. Laccase activity was found to be maximum with the inoculum size of 200 μ l.
- All the metal ions used in the present study supported growth and influenced laccase production. Among the metal ions ferric chloride gave maximum growth and laccase production.
- The selected surfactants were found to support the bacterial growth. Among the surfactants studied Triton X-100 was found to yield the best laccase activity.
- According to Plackette- Burman design ammonium sulphate did not exert any effect on bacterial growth and laccase production.
- The inoculum size, sodium acetate, yeast extract and agitation were found to have positive effect on both laccase production and bacterial growth.
- Magnesium sulphate, incubation period, phenol and trace element were found to have negative impact on both laccase production and bacterial growth.

- The pH was found to have positive influence on enzyme production and negative influence on growth whereas glucose was found to have negative influence on enzyme production and positive influence on growth.

Conclusion

The selected medium components were optimized by one time one factor approach for the isolated bacterial strain. Plackette -Burman Design was performed for further optimization of bacterial growth and laccase activity. The conditions optimized for laccase production from the isolated bacterial strain could be selected to obtain large amounts of laccase. This could be exploited for various biotechnological applications.

Suggestion for Further research

- ✓ The Response surface methodology can be adopted for further optimization of laccase production.
- ✓ Bioremediation efficiency of the optimized laccase can be assessed.
- ✓ Gene encoding laccase can be studied in detail