Screening of Mushrooms from Exotic Locales for Laccase Production: Studies on the Enzyme and the Application of Immobilized Laccase on dye Degradation

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ABSTRACT

The pollution problems due to the textile industry effluents have increased in the last years. From the available literature it can be estimated that, the azo dyes are the most important chemical class of synthetic dyes. The degradation products of textile dyes are often carcinogenic. The idea of pretreatment of effluents released from textile industry with laccase enzyme before releasing it to water bodies will be an excellent solution for this serious health hazard.

The present study focused on screening of various mushrooms collected from exotic locales for laccase production and to find out the best production strain among the collected mushrooms. 8 differenet mushroom strains were collected. Mushroom strains collected from various exotic locales includes Sample 1(Identified as *Termitomyces* sp from Ambanad hills (Kollam), Sample 2(Ponmudi), Sample 3(Nedumangad- Trivandrum) , Sample 4(Kollam), Sample 5(Medical college-Trivandrum), Sample 6 (Idukki), Sample 7 (Pazhayarode- Trivandrum), Sample 8(Vattappara-Trivandrum). Out of the 8 strains only 4 strains (Sample1, 2, 6 and 7) turned positive for laccase production. The 4 selected strains were analysed for laccase production in submerged as well as solid state fermentation after optimizing the pH of the growth media. Among the 4 selected strains, sample 1 showed excellent results. The molecular identification of the selected strain 1was done by 18s rRNA amplification using ITS primers, ITS1(Forward) and ITS 4 (Reverse). The 650 bp amplicon obtained was sequenced and BLAST analysis was perfomed. The sequence showed 99% similarity with *Termitomyces* sp. with a query coverage of 98%. *Termitomyces* sp(Sample1 was selected for further studies including partial purification, laccase immobilization and dye degradation studies.

Partial purification through ammonium sulphate precipitation showed maximum activity in 60% ammonium sulphate fraction. The extent of purification was analyzed by doing SDS-PAGE. Activity

staining of non reducing SDS gel showed the presence of heterodimer having molecular weight of ~60kDa(Band 1) and 40 kDa (Band 2). Enzyme characterization of partially purified laccase was performed. Optimum temperature of laccase enzyme was found to be 50°C and optimum pH was found to be 4.

The partially purified enzyme preparation from SSF was used for immobilization studies. Preliminary studies of dye degradation with crude laccase showed good results. The rate of decolorization was found to be maximum in the first 24 h. A 75% decrease in absorbance was observed when ~ 25 U of crude laccase preparation was treated with 5 mM methyl orange. This indicates the potent ability of the isolated laccases to degrade 75% of the dye in 24 h.

For laccase immobilization, entrapment method using calcium alginate was used. The major problem in using calcium alginate beads is the stability and shelf life of the beads for reusage and recycling in experimental studies. The present study focused to improve the stability of calcium alginate beads by trying a combination of pectin or egg shell powder to the molten and cooled sodium alginate-enzyme mixture before making it into calcium alginate beads.

The enzyme activity of laccase immobilized calcium alginate beads was found to be 0.8 U/10 mg (1st set)of beads and that of calcium alginate-pectin combination is 0.17 U/10 mg of beads. Calcium alginate-egg shell powder combination showed an enzyme activity of 0.6 U/10 mg of beads. Calcium alginate-pectin beads started disintegrating at acidic pH 4 (buffer) provided in the experiment. This might be the reason for the increase in absorbance compared to the control. Hence we dropped the experiment with laccase immobilized in calcium alginate- pectin beads. The influence of pH on the stability of pectin beads should be an objective for future studies.

The addition of egg shell powder prevents even shaped bead formation due to clump formation of egg shell powder in sodium alginate- enzyme mixture. There was no significant increase in enzyme activity and stability of calcium alginate beads. The ability of the immobilized laccase in dye degradation was analysed using 5mM methyl orange solution with 3g (~250U) of immobilized laccase. The result observed was good. An ~ 50% dye degradation was observed even in the first six hours and more than 80% of dye got degraded in 24 hours of enzyme treatment. The stability of the beads was analysed by reusing the beads for a second trial after 2 weeks. But the result was not promising as the bead texture got changed and the beads got degraded to a greater extent in a two

week time span. Further optimization and pretreatment studies need to be conducted before using egg shell powder to enhance the stability of calcium alginate beads.

Key words : Laccase, Dye- Decolorization, Immobilization, Calcium Alginate

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