4/4/07/04/2017

Name of Scholar: **SUMBUL AFREEN**

Name of Supervisor: **Prof. Tasneem Fatma**

Name of Co- Supervisor: **Dr. Nadeem Ahmad**

Department: **Department of Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi**

Title of the Study: **Detection And Characterization Of Laccase From Cyanobacteria**

Abstract

Anthraquinone dyes are second most important class of synthetic dyes and are widely used in paper and pulp mills, textiles and dyestuff industries, distilleries, and tanneries industries, which release highly colored waste waters. Effluents containing dyes are highly colored and are usually the first contaminant to be recognized in waste water. Disposal of the untreated dyeing effluent in water bodies causes serious environmental and health hazards. Various physicochemical methods, like adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis, photodegradation for the remediation of dye wastewaters but major disadvantages of this methods are high cost, low efficiency, limited versatility and the handling of the waste generated. Biological methods are generally considered environmentally friendly as they can lead to complete mineralization of organic pollutants at low cost. Various microorganism such as fungi, yeast, bacteria, algae and their enzymes have potential to decolorize textile dyes under certain environmental conditions .

Cyanobacteria (blue-green algae) are ubiquitous in nature and have enormous ability in the treatment of industrial effluent and waste water. Some species of cyanobacterial such as *Anabaena variabilis, Oscillatoria salina, Nostoc muscorum* and *Lyngbya majuscula* were studied and compared for effective removal of the pollutants from textile industry effluent but enzyme involved in this process has not been characterized. In the case of enzymatic remediation of synthetic dyes, laccases seem to be the most promising enzymes. Laccase (EC 1.10.3.2) belonging to the multicopper oxidases family, generally extracellular and catalyzes the oxidation of several phenolic compounds, aromatic amines, thiols and some inorganic compounds using molecular oxygen as electron acceptor. The low substrate specificity makes this enzyme

interesting for biotechnology purposes in various industries such as pulp and paper and textiles and bioremediation of industrial pollutants. Due to advantage of cyanobacteria to oxygenate the environment, short generation time and easy mass cultivation, it may used as a good source for laccase production.

So, In the present study 35 heterocystous and non heterocystous strains of cyanobacteria has been investigated for laccase production. Among all selected strain 29 strains showed the positive results and *Spirulina platensis* CFTRI gave the highest laccase activity. The laccase production was found to be maximum on 10^{th} day under normal growth condition. The average time of laccase production was decreased to 4^{th} day in presence of inducer guaiacol. The combination of (2,5-Xylidine + guaicol) act as best inducer that enhanced the laccase production 36.2 folds (582.92 Uml^{-1}) due to synergistic effect. The optimum condition for laccase production was 30°C & pH 9, glucose as carbon source and sodium nitrate as nitrogen source.

A novel extracellular laccase enzyme produced from *Spirulina platensis* CFTRI was purified by ultrafiltration, cold acetone precipitation, anion exchange and size exclusion chromatography with 51.5% recovery and 5.8 purification fold. The purified laccase was a monomeric protein with molecular mass of ~66 kDa that was confirmed by zymogram analysis and peptide mass fingerprinting. The optimum pH and temperature of the enzyme activity was found at 3.0 and 30ÊC using ABTS as substrate but the enzyme was quite stable at high temperature and alkaline pH. The laccase activity was enhanced by Cu^{+2}, Zn^{+2} and Mn^{+2}. In addition, the dye decolorization potential of purified laccase was much higher in terms of extent as well as time. The purified laccase decolorized (96%) of anthraquinonic dye Reactive blue- 4 within 4 h and its biodegradation studies was monitored by UV visible spectra, FTIR and HPLC which concluded that cyanobacterial laccase can be efficiently used to decolorize synthetic dye and help in waste water treatment.