ABSTRACT

Lignin is the second most abundant constituent of the cell wall of plants, where it protects cellulose towards attack by different pathogenic microbes and chemicals. Its successful exclusion is a key step for utilization of plant biomass for industrial purposes. Laccases (EC 1.10.3.2) are a class of extracellular enzymes which oxidize the phenolic groups in lignin.

In this investigation, yellow laccase has been used for the degradation of lignin of *Ricinus communis* (palm of Christ). Optimization of yellow laccase production was carried out by *Lentinus squarrosulus* (MR13) (commonly known as white rot fungi) a locally isolated strain of IIT, Kharagpur campus. Different physico-chemical factors that influence the secretion of extracellular enzyme were selected for yellow laccase production under solid state fermentation. Preliminary studies for screening of important parameters were carried out using one variable at a time approach which was followed by statistical and computational optimization techniques such as Response Surface Methodology (RSM) and Genetic Algorithm (GA). The maximum yellow laccase yield was recorded as 26,934 IU gds^{-1}, when incubation time 7.9 days, solid to liquid ratio1:1.85, size of rice straw 1.4 cm, 32 °C, pH 5.9, inoculum size 5.9 % and surfactant concentration 0.97 %.

Purification and characterization of yellow laccase was carried out to gain insights on the properties of the enzyme. It was revealed that the enzyme was able to retain its activity in a long range of pH, from 4.0 to 9.0. At extreme pH i.e., 3.5 and 9.5 enzyme was able to retain its half activity. The optimum temperature of yellow laccase activity was obtained at 40 °C. The enzyme under evaluation is considered to be stable at higher temperature. The half life of yellow laccase was found to be 27.5 h at 40 °C.

The crude enzyme was then used for the degradation of lignin of *Ricinus communis* and optimization was carried out using one variable at a time approach followed by RSM and GA. The effects of several parameters such as pH, temperature, enzyme concentration etc. were investigated. It was found that under optimum conditions i.e. are incubation time 6.4 h, pH 6.82, 42.8 °C, solid to liquid ratio 1:2.7 and enzyme activity 655.9 IU mL^{-1}, 86.7 % lignin was degraded. Identification of lignin degraded products were also carried out using GC-MS which indicated the presence of several low molecular weight aromatic compounds. These aromatic compounds released

during enzymatic lignin degradation, can be effortlessly connected to the oxidization of the sinapylic and coniferylic alcohol because these are considered to be the fundamental moieties that build the lignin.

Key words: Yellow laccase, *Ricinus communis*, response surface methodology, genetic algorithm, solid state fermentation.