ABSTRACT

2,4-DCP poses a serious threat to the environment of a region due to its cytotoxic and suspected genotoxic effects. Many physicochemical and biotechnological techniques have been used for the degradation of 2,4-DCP but they have not met with much success at the ground level. The physicochemical methods are cost intensive and the existing biotechnological methods, which employ whole cells and are time consuming, become ineffective above a certain concentration as the cells responsible for degradation fail to thrive themselves.

In the present investigation, a new biotechnological technique for the degradation of 2,4-DCP has been devised. The lignolytic enzyme laccase has been used for the biodegradation of 2,4-DCP, exploiting the structural homology of 2,4-DCP with lignin, the natural substrate of laccase. In order to make the process economically feasible, optimization of laccase production was carried out from a locally isolated hyperactive strain of *Pleurotus* sp. taking into consideration different factors such as particle size, pH, moisture content etc. which influence production. Initial studies were carried out using one variable at a time approach which was followed by statistical and computational optimization techniques such as RSM and GA which gave a laccase yield of 52594 IU gds^{-1}. Purification and characterization of laccase was carried out to gain insights on the properties of the enzyme. It was revealed that the crude enzyme contains two isozymes which vary widely in their isoelectic points (pI 3.8 and 9.2).

The crude laccase was then used for the biodegradation of 2,4-DCP 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, initial enzyme concentration were investigated and several other factors that influence the degradation such as concentration of heavy metals and inhibitors were evaluated. It was found that in the optimum conditions ~99 % of 2,4-DCP at 1 mM concentration was degraded within a span of 8 h. Evaluation of kinetic parameters revealed a K_m value of 200 mg L^{-1}. Identification of the intermediates were also carried out using HPLC and LC-MS which indicated the oxidation of the phenol ring and formation of low molecular weight organic acids which can be easily mineralized by the microorganisms. Further economization of the process was attempted by immobilization of laccase in semi-interpenetrative network of copper alginate and acrylamide which gave an immobilization yield of ~99 % and a reduction in the K_m value.

Keywords: Laccase; 2,4-DCP; biodegradation; response surface methodology; genetic algorithm; isozymes; semi-interpenetrative network.