STUDY ON A MODIFIED LACCASE FOR LACQUER INDUSTRY

Ph.D DISSERTATION

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ABSTRACT

Laccase enzyme was extracted from thitsi (Myanmar lacquer) and purified by 3 purification steps. First, partially purified laccase was obtained by ammonium sulphate precipitation method, the purification fold was 5 times greater than with respect to crude laccase. Then, applying on cation exchange chromatography enhanced the purity of laccase with specific activity of 11.8 EU mg^{-1} with a purification fold of 7 times greater than the crude extract. Finally, the third purification step of using DEAE Sephadex A-50 anion exchange resin was found to produce an effective purified laccase with specific activity of 13.4 $EUmg^{-1}$, which was 8 times greater than that of crude laccase. The overall percent of purified laccase with respect to the crude laccase was 21%. Copper content in purified laccase was 0.21 weight %. The molecular weight of purified laccase estimated by non SDS. PAGE gel electrophoresis test was 1.2×10^5 Da. The protein content of purified laccase was found to be 0.3 $mgmL^{-1}$. Then, the activity and stability of purified laccase were enhanced by modification with anionic polysaccharide carboxymethylcellulose. The optimum enzyme performance of CMC modified laccase was achieved at specific conditions for modification reaction, at 30°C, at the pH of 6.0, when the enzyme to modifier weight ratio was 1:4 and 48 hours of incubation time. CMC modified laccase activity was about 1.3 times that of purified laccase. CMC modified laccase displayed higher catalytic activity along with enhanced thermal stability. The T_{50} for purified laccase was 40°C and T_{50} for CMC modified laccase was 70°C. Optimal pH range from 4 to 8 was obtained for CMC modified laccase and at all pH values, the activity of CMC modified laccase was higher than that of purified laccase. Kinetic parameters of purified laccase and CMC modified laccase were determined by using guaiacol oxidation reaction, K_m values were 3.43 mM and 3.02 mM for purified laccase and modified laccase respectively. CMC modified laccase possess higher affinity of substrate than the purifed laccase. The results

and data showed that when laccase enzyme subjected to modification by means of covalent coupling using soluble polymer CMC results in enzyme efficiency which retain high biological activity and thermal stability within a temperature. Thus, it was employed as a coating material in Myanmar lacquerwares. Addition of CMC modified laccase to lacquerware coating liquid thitsi, effectively reduced the drying time as well as to form a uniform consistant adhesive thitsi coating. CMC modified laccase with the lacquer coating using the Phar-un thitsi dried between 7 hours 30 minutes and in the case of Moe-nai thitsi dried completely in 3 hours 15 minutes. The reduction in drying time of lacquer coating may be attributed to the enhanced laccase activity of CMC modified laccase which may be accounted to be 10 to 11 times that of the crude laccase in the thitsi. Therefore, modification of laccase with macromolecular substance carboxymethyl cellulose may have contributed significantly an effective drying process of using thitsi in Mynamar lacquerware coating to create uniform lustre with aesthetic qualities.

Key words : CMC modified laccase, enhanced activity, pronounced thermal stability, lacquerware coating, lacquer Industry.